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FURTHER STUDIES ON THE OPALINID CILIATE INFUSORIANS AND THEIR HOSTS

By

MAYNARD M. METCALF

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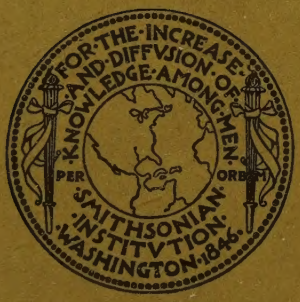

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FURTHER STUDIES ON THE OPALINID CILIATE INFUSORIANS AND THEIR HOSTS

By MAYNARD M. METCALF

INTRODUCTION

THIS paper should be read in connection with "The Opalinid Ciliate Infusorians," U. S. National Museum Bulletin 120, 1923, of which it is really a revision and a second part. About 30 new species and subspecies are described; species described by others since 1923 are considered and illustrations and measurements copied; the taxonomy of the family is reviewed, as well as the data and hypotheses as to geographic distribution; and former reviews of the literature (Metcalf, 1909 and 1923a) are brought to date. Thus, Bulletin 120 and the present paper together cover the family Opalinidae as now known.[1]

The whole body of data as to geographical distribution of the parasites and the hosts is discussed in an attempt to answer a number of questions as to, e. g., place of origin of each of the several families, subfamilies, and some of the genera of Anura; the geologic period of

[1] The cytology of the opalinids described in the present monograph has not been studied in detail. The author welcomes and cordially subscribes to the findings and conclusions of his friend Dr. T. T. Chen, who, in a series of studies, has described the behavior of opalinid chromosomes during mitosis in greater detail than has been done previously. Dr. Chen has demonstrated for the first time for the opalinids the following: (1) Individuality of chromosomes; (2) diploidy; (3) the relation between chromosomes and nucleoli; (4) that the so-called "macrochromosomes" described by other investigators are not chromosomes or a distinct set of chromosomes different from the ordinary kind but parts of certain chromosomes; (5) that the so-called "midmitotic resting stage" described by other investigators is a misinterpretation, the nucleoli in the resting nucleus having been considered as chromosomes. These phenomena had escaped observation or proper interpretation by other workers, though some of them were working in the best laboratories for protozoan cytology. I have seen Dr. Chen's preparations, and I am very glad of this opportunity to refer to the skill and accuracy of his beautiful studies, which place the cytology of the opalinids on a new and sound basis.

the origin of each group of these hosts; the routes and the geologic times of the distribution of several groups of hosts; the places and times of origin of the several genera and some of the subgenera of opalinids and the routes and times of their distribution. Paleogeographic hypotheses of Arldt, Haug, Scharff, Schuchert, and others are tested by using them in connection with the distributional data from Anura and opalinids and seeing whether the hypotheses furnish reasonable explanations of the faunal data. The methods of speciation in the opalinids and the general principles of their evolution also are discussed.

ACKNOWLEDGMENTS

I have received invaluable assistance of various sorts from many sources during the course of these studies. Many institutions have contributed to the work: The Johns Hopkins University, before I was elected to the faculty, welcomed me for a year to its department of zoology and for another year to its school of hygiene; the United States National Museum gave permission to gather opalinids from its anuran collections, Dr. Leonhard Stejneger aided with many suggestions, and Miss Doris Cochran assisted by identification of species, especially of tadpoles from India and Burma; Prof. T. N. Annandale and the Indian Museum at Culcutta sent numerous Indian Anura, as also did the Colombo and Madras Museums; the National Academy of Sciences helped, with a grant of money and with introductions, toward a half-year trip to South America for collecting and study, and for this trip Prof. Vernon Kellogg, executive secretary of the National Research Council, Dr. C. D. Walcott, secretary of the Smithsonian Institution, and Dr. Leo S. Rowe, director general of the Pan American Union, gave most helpful introductions; the Oswaldo Cruz Institute, especially Prof. Adolpho Lutz, Miss Bertha Lutz, and Dr. Gualter A. Lutz, obtained for me fine collections of living Anura from Rio de Janeiro and several neighboring Brazilian states and furnished me luxurious laboratory facilities; the Institute of Hygiene and Public Health of the University of Montevideo rendered similar service; the Marine Biological Laboratory of Woods Hole, Mass., furnished much material, including a large series of tadpoles of *Rana clamitans* in all stages of development; the Zoological Museum at Ann Arbor, Mich., through the kindness of Prof. A. G. Ruthven and Mrs. Helen T. Gaige, gave a complete series of larvae of *Ascaphus truei*; Prof. C. E. McClung and Dr. C. L. Parmenter, of the University of Pennsylvania, proffered the hospitality of their vivarium for about two years to a hundred or more specimens of *Bombina igneus* and *B. pachypus*, though unfortunately the fire toads did not breed.

Many individuals also have helped greatly with material or data or both: Prof. W. A. Haswell and the late Prof. Launcelot Harrison

(data on Australian Anura); Prof. W. B. Benham, of Dunedin, New Zealand (specimens of the very rare *Liopelma* and data as to its habits and development); Prof. G. E. Gates (Anura from Rangoon, Burma); Prof. Robert Hegner (Anura from the Philippine Islands); Dr. Ergastri Cordero, of Montevideo, and Dr. Carlos Porter, of Santiago de Chile (South American Anura and data); and Prof. E. V. Cowdry (two specimens of the very rare *Heleophryne regis*). Miss Margaret Cowles (Mrs. Wilson Shaffer), of Johns Hopkins University, has worked through the life history of *Opalina virguloidea* in tadpoles of *Rana sylvatica*, as well as helping in the preparation of some of the South American material. I am also indebted to Mrs. Lura Carper, of the zoological laboratory of the Johns Hopkins University, for revising the bibliography, and to Mrs. Caroline Hutzler Bernstein for copying several drawings of opalinids used.

For all this assistance from all sources and for the many personal kindnesses accompanying it I take this opportunity to express most grateful appreciation.

DESCRIPTIONS OF SPECIES AND NEW DATA AS TO HOSTS AND DISTRIBUTION [2]

Genus PROTOOPALINA Metcalf

PROTOOPALINA APPENDICULATA Fantham

FIGURE 21

Host: *Rana fuscigula* Duméril and Bibron, from Johannesburg, South Africa.

FIGURE 21.—*Protoopalina appendiculata* Fantham, × 470. (After Fantham)

This is a distinct and very interesting species, chiefly because of the marked tail. An elongated, slender, posterior end is characteristic of numerous species of *Protoopalina*. In all of them the cilia are long at the anterior end of the body, and they usually diminish in length and in number toward the posterior end, which is free of cilia. See p. 559 for a discussion of the comparative structure of the posterior ends of different species in the several genera.

Fantham's specimens measured, in microns: Length, 87–136; width, 22–51. Nucleoli are described as 2 and 4 in different indi-

[2] The drawings that illustrate this section are generally incomplete; for example, usually only a few of the cilia are drawn, or but few of the nuclei in the multinucleate species; only few of the lines of cilia are indicated and these only partially. Only enough is shown to give the features used in diagnosis of the species. A pair of dots outside the contour of the body in the drawings when found indicate the limits of the morphologically anterior end.

viduals. Mitosis was not described, and in consequence the number of nucleoli is not definitely determined.

PROTOOPALINA BIBRONII, new species

FIGURE 22

Type: U.S.N.M. No. 22621.

Host: *Pseudophryne bibronii* Günther, four infections from Australia as follows: U.S.N.M. No. 10968 from the Paris Museum; No. 63181 from Port Lincoln, Kangaroo Island; and two, Nos. 64056 and

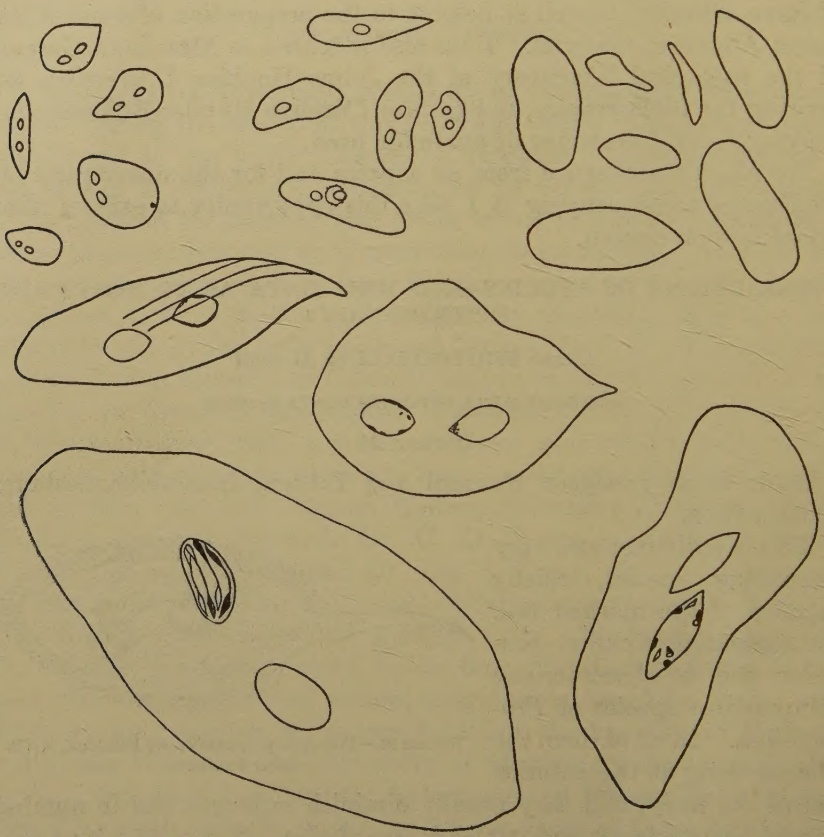

FIGURE 22.—*Protoopalina bibronii*, new species: Upper three groups of figures, × 146; other four, × 673.

64057, from Ehor, New South Wales; these and the Port Lincoln specimen collected by Hoy.

Measurements in microns: Body, 80 by 25 (average specimen), 60 by 37 (wide form), 67 by 20 (narrow form); nuclei, 21 by 6, 17 by 8, 11 by 6, 9 by 6; cilia line interval anteriorly 2, posteriorly 3. Nucleoli number 4. The infection in frog No. 63181 (fig. 22, upper left group) shows many broad, flat forms, with regions of the protoplasm abnormal, the animals containing generally a huge, irregular, lateral vacuole. Had these abnormal, partly degenerate, usually flattened forms been

the only ones found, they would probably have been thought to be Zelleriellas. Indeed I so labeled the unstained specimens. Probably the host was dead some time before it and the parasites were preserved.

This species is very distinct from any of the other Australian species described. It resembles *P. intestinalis* and *P. hylarum* and is classed with them in the subgeneric group II, which contains the parasites characteristic of the bell toads.

PROTOOPALINA BORNEONENSIS, new species

FIGURE 23

Type: U.S.N.M. No. 22622.

Host: *Polypedates reinwardtii* (Boie), U.S.N.M. No. 57819, from Borneo.

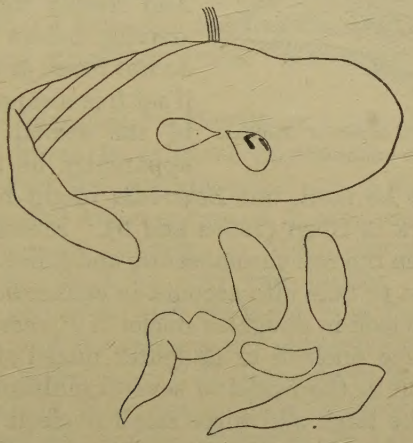

FIGURE 23.—*Protoopalina borneonensis*, new species, × 124 and 505.

Measurements, in microns: Large specimen, 200 by 36; medium individual, 140 by 40; small specimen, 89 by 30; nucleus of medium individual, 17 by 8.5; cilia length, 9.1; cilia line interval in posterior half of body, 4.1. This species somewhat resembles *P. africana* Metcalf.

"PROTOOPALINA CACCOSTERNI" Fantham

FIGURE 24

Host: *Cacosternum boettgeri* (Boulenger), tadpole, from Johannesburg, South Africa.

Measurements, in microns: Length of body, 37.3–63.6; width of body, 6.4–11; length of nucleus, 6–8; width of nucleus, 2–3. Nucleoli "3" in each end of the nucleus when found in an anaphase of mitosis.

This odd number of what appear to be daughter nucleoli seems inconsistent with the derivation of half the number of nucleoli from the male and half from the female, and observation of the sexual phenomena in several species of opalinids seems to have shown that the male

and the female gamete each contribute the same number of nucleoli and that the zygote contains the double number. In several other species of opalinids an odd number of nucleoli seems to be present. How shall the puzzle be resolved? Chen (1936b) has shown that when two or more nucleoli are close together they may fuse into a single body. This offers a plausible explanation.

I have tried for a number of years to get material for restudy of the sexual phases of the life-history in some *Protoopalina*, preferably the Protoopalinas of *Bombina*, the fire toad, either *P. intestinalis* or *P. caudata*, but repeated attempts have failed. The Bombinas, although successfully imported and living for more than a year in vivaria, do not breed. When freed by the dozens into apparently suitable environments they are not seen again. And freshly imported specimens, arriving before the eggs are laid, do not breed or even copulate, and if set free disappear. The restudy of the sexual phenomena must apparently be done in Europe if *Protoopalina* is to be used, but *Zelleriella* might be better as shown by the recent work of Chen (1936a and b). Metcalf (1909) failed to distinguish between the real chromosomes and other nuclear structures.

FIGURE 24.—*"Protoopalina caccosterni"* Fantham, × 100. (After Fantham.)

Items of interest to take into account in connection with Fantham's report of three nucleoli in daughter nuclei of *P. caccosterni* are: (a) The appearance of three nucleoli in daughter nuclei of *P. ovalis* (fig. 30) and of five nucleoli in the nuclei of several multinucleate species; (b) the presence of one large and three small nucleoli in each nucleus of *P. axonucleata lata* (see Metcalf, 1923a), the nucleoli not being in pairs, and of *P. meridionalis* (fig. 28); (c) the presence of one large nucleolus and a chromatin skein in the nuclei of cysts and of young forms of *Opalina chattoni* (Weill, 1929); (d) the presence of one large nucleolus and of scattered small chromatin granules in nuclei of adults of *O. nucleolata* (p. 538); (e) the transverse division of nucleoli in *Protoopalina intestinalis* and *P. caudata* at about the time of the ill-defined equatorial plate stage (Metcalf, 1909), and the appearance of longitudinal splitting of the nucleolus in the telophases of the same nuclei (Metcalf, 1909, cf. Konsuloff, 1922); [Are the apparently daughter nucleoli in the anaphases and telophases in *Protoopalina* really double?] (f) the origin of the nucleoli in the postsexual stages of the life history. These and other items should be studied.

We should realize that *"P. caccosterni"* is reported only from tadpole hosts. It may not be a *Protoopalina* at all but may be the *Protoopalina*-stage in the development of a *Cepedea* or an *Opalina*, though this is very unlikely, since no individuals with more than two

nuclei are reported and tadpoles naturally infected would probably show parasites of different ages, the older of which would be multinucleate if the adults were *Cepedea* or *Opalina*.

PROTOOPALINA [CAPENSIS, new species]

Host: *Heleophryne regis* Hewitt.

Through the kindness of Dr. E. V. Cowdry I obtained two specimens of this very rare and extremely interesting little frog, collected by Dr. John E. Rex at Eastford, Krupna, Cape Province, South Africa. They were preserved in formalin and after some weeks were transferred to alcohol, not a satisfactory method of preservation, since formalin allows deterioration of the opalinids. One frog, 44 mm. long, now deposited in the U. S. National Museum as No. 67842, contained numerous Protoopalinas, slenderly pointed behind and belonging evidently to what I have described as the most archaic subgeneric group of this most primitive genus. The other frog showed no opalinids.

Heleophryne was regarded by Hewitt as a leptodactylid, and such it appears to be from its external appearance and its arciferous shoulder girdle. But the leptodactylids are a family of southern South American origin, which colonized Australasia, entering by way of Antarctica. They are unknown in Africa except for *Heleophryne*, which is represented there by only two species. Is *Heleophryne* a true leptodactylid and, if so, how did it get from Patagonia to Africa? In South America and Australasia the characteristic opalinid of the Leptodactylidae is *Zelleriella*. If *Heleophryne* in South Africa carried *Zelleriella*, an opalinid that was evolved in South America in the Leptodactylidae and was carried wherever the Leptodactylidae have spread, it would clinch the evidence for the leptodactylid nature of *Heleophryne* and its origin from South American ancestors. It was this consideration that led Professor Cowdry to undertake to get for me specimens of *Heleophryne*.

Zelleriella was not found, but rather a species of a more archaic group, representative of which are found in Patagonian and Australasian leptodactylids, in Papuan Hylas, and in tropical African Pipidae (Xenopodinae), and so the question of the origin and relationships of *Heleophryne* is still open. Its parasite is consistent with *Heleophryne*'s origin from a South American leptodactylid but does not clinch this hypothesis as finding *Zelleriella* would have done. If such was the origin of *Heleophryne*, the spread from Patagonia to Africa was later than the origin of leptodactylids in Patagonia, and this was later than the separation of Australasia from Asia in the early Cretaceous period, probably considerably later. Interpretation of *Heleophryne* as a leptodactylid indicates connection between South America and Africa at least as late as the middle Cretaceous period.

Dr. Stejneger, however, has expressed doubt of the leptodactylid affinities of *Heleophryne*, in spite of its resemblance, suggesting that it may well be a ranid in a state of arrested development. Its parasite throws no light upon this suggestion.

I am unable to give illustrations of the *Protoopalina* from *Heleophryne*, to which when found I gave the provisional name of "*capensis*, new species." In some way, not understood, during my several years of illness the material has disappeared. It was of such unique interest that I separated it from the material of about 30 other species still to be studied, and placed it so carefully away that with all my searching it has not reappeared. Apparently moving my laboratory and getting settled in a new place, when I was too ill to give it proper attention, led to the disappearance of this especially prized material. The provisional name must not be accepted without more adequate description and the preservation of a type specimen. I gave it only for convenience of reference. The fact of the infection of a specimen of *Heleophryne regis* by a *Protoopalina* of the most primitive subgenus is, however, definitely recorded.

PROTOOPALINA CAUDATA MICROHYLA Nie

FIGURE 25

Host: *Microhyla ornata* Duméril and Bibron, Indian Mus. No. 17287, sent by Professor Annandale; collected at Harnai, Ratnagiri District (south of Bombay), among mountains.

Measurements, in microns: Body, 147.7 by 43.3; nucleus, 14 by 8.5; cilia length, 9.7. Apparently 6 (?) nucleoli.

This form resembles *P. caudata*. Its posterior end is slightly pointed in a few specimens. Its measurements are about as in *P. c. discoglossi*.

PROTOOPALINA DORSALIS (Raff)

FIGURE 26

Host: *Limnodynastes dorsalis* (Gray). I have had one good infection from a frog 54 mm. long (U.S.N.M. No. 64043) from Busselton, Western Australia, collected by C. N. Hoy, June 8, 1920.

Measurements, in microns: Body, 240 by 60, 100 by 68, 180 by 33; nucleus, 25 by 8.9; cilia length, 9.8. Nucleoli, 4.

My specimens belong apparently to Raff's species. They are somewhat intermediate in appearance between *P. caudata* and *P. intestinalis*. I am therefore placing *dorsalis*, along with *P. peronii*, in group II with them.

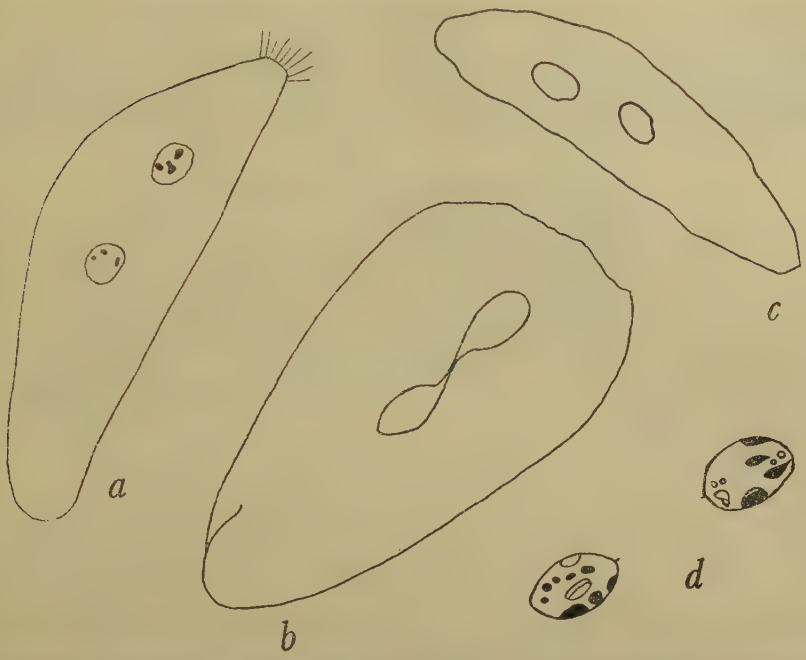

FIGURE 25.—*Protoopalina caudata microhyla* Nie: *a–c*, × 460; *d*, × 1010; *b*, a daughter cell just from transverse division (?); *d*, nuclei of an ordinary individual.

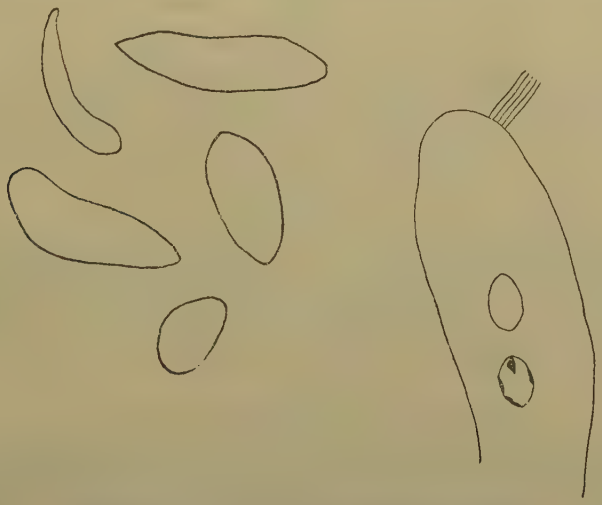

FIGURE 26.—*Protoopalina dorsalis* (Raff), × 117 and 505.

PROTOOPALINA LUZONENSIS, new species

FIGURE 27

Type: U.S.N.M. No. 22624.

Host: *Kaloula picta* (Eydoux and Souleyet), a gastrophrynid, from Luzon, Philippine Islands, U.S.N.M. No. 57758, 32 mm. long, very abundant infection.

Measurements, in microns, for some individuals: Body, 313 by 80, 70 by 23; nucleus, 19 by 19, 20 by 15; cilia length, 9.

The widely separated nuclei with no connecting thread help to distinguish this very long species. *P. hylarum* (Raff) has this same

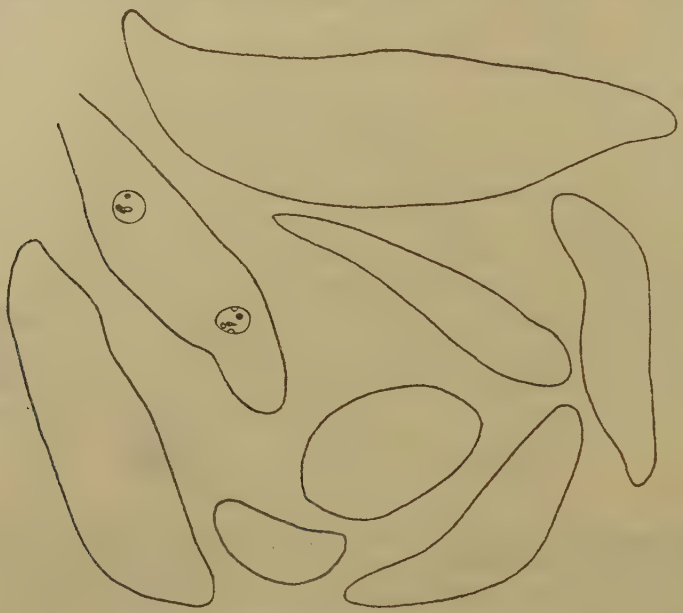

FIGURE 27.—*Protoopalina luzonensis*, new species, × 249.

feature, but it is nearly twice as large (linear measurement) and its nuclei are smaller. The number of nucleoli is undetermined.

PROTOOPALINA MERIDIONALIS Fantham and Robertson

FIGURE 28

Host: *Rana delalandii* (Tschudi), from Johannesburg, South Africa.

Measurements, in microns (given by Fantham): Body length 80–240, width 24–80; nuclei length 10–20, width 7.5–12.5. Note the large nucleolus in each of the nuclei in figure 28, *b* (cf. the dimensions under *P. caccosterni*).

PROTOOPALINA MOSSAMBICENSIS Metcalf (?)

Fantham found what he regards as this species in the same host, *Rana adspersa* Tschudi, from Johannesburg, South Africa. The measurements, in microns, he gives are: Body, 127 by 50. He reports the nuclei as "spherical to ellipsoidal." The specimens described by Metcalf (1923a) from Mozambique have slender, spindle-shaped nuclei even in the cysts, though one cyst is drawn with an unusually large, nearly spherical nucleus. In the adults Metcalf found the slender nuclei united always by a thread. The specific identity of the Johannesburg and the Mozambique forms seems doubtful.

PROTOOPALINA NYANZA Lavier

Host: *Varanus niloticus* Linnaeus, from the shores of Lake Victoria Nyanza. This is probably an adventitious, temporary infection due to the host having eaten the natural, anuran host. It is unlikely that

FIGURE 28.—*Protoopalina meridionalis* Fantham and Robertson: *a*, × 350; *b*, three nuclei, × 400. (After Fantham.)

a lizard would have aquatic breeding and feeding habits that would allow propagation of its opalinid parasites, at least without an intermediate host.

Lavier gives no drawings. The chief points on his description are as follows: Elongated, circular in cross section or some individuals a a little flat; length of body 88–212μ, width of body 20–43μ; nucleus length 13–20μ, width 8–12μ; posterior spine 8–9μ long. The resting nucleus shows 4 nucleoli. A unique (?, see Leger and Duboscq, 1904, *Protoopalina saturnalis*) band of nucleolar substance occupies three-quarters of the equator of the spindle and makes it difficult to count the individual nucleoli. Anterior cilia 22–25μ long, the posterior ones grading down to a length of 6μ; cilia line interval 3μ in front, 3.5μ behind. The whole length of the body is ciliated except the posterior spine. The oval endospherules are 1–2μ.

PROTOOPALINA OCTOMIXA Fantham

FIGURE 29

Host: *Bufo carens* A. Smith, from Johannesburg, South Africa.

Measurements given, in microns: Body length 175–425, width 55–140; nucleus length 26–36, width 18–27. Nucleoli 4.

PROTOOPALINA OVALIS Fantham

FIGURE 30

Host: *Rana fuscigula* Duméril and Bibron, from Johannesburg, South Africa.

Measurements, in microns: Body length, 76–156.3, width, 30.8–84; nucleus length, 10–19, width, 4.5–14. Nucleoli 6 in large individuals,

FIGURE 29.—*Protoopalina octomira* Fantham: *a*, × 75; *b*, a nucleus, × 800. (After Fantham.)

3 in daughter cells with their nuclei recently from division (Fantham's fig. 6) (see dimension under *P. caccosterni*).

Fantham figures an arrangement of the lines of cilia that differs markedly from any I have ever found in any opalinid. In all other

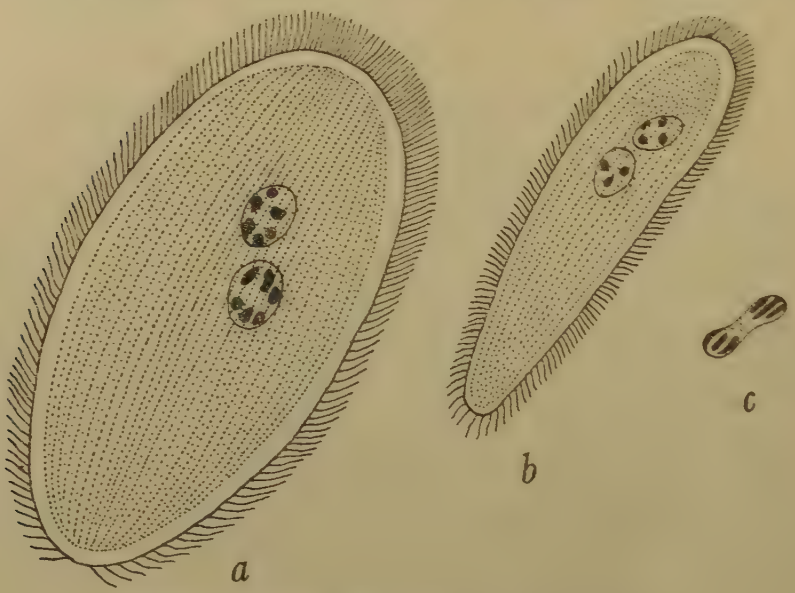

FIGURE 30.—*Protoopalina ovalis* Fantham, × 470. (After Fantham.)

species studied the lines are parallel and spiral. Fantham shows them as converging to the two ends of the body. It would be well to reobserve these animals as to this feature. The lines of cilia were not discovered in my rather poorly preserved *P. ovoidea* (Metcalf, 1923a).

PROTOOPALINA STEJNEGERI Metcalf

FIGURE 31

Host: *Ascaphus truei* Stejneger, from the Olympic and Siskiyou Mountains in extreme Northwestern United States.

For the sake of having all known opalinids at least briefly mentioned in U. S. National Museum Bulletin 120 or in this paper, which is

really a second part of that bulletin, I am copying some of the figures and part of the data from my former descriptions published elsewhere.

Measurements, in microns: Length of body 170 (large), 124 (medium), 62 (small); width of body 24 (large), 23 (medium), 17

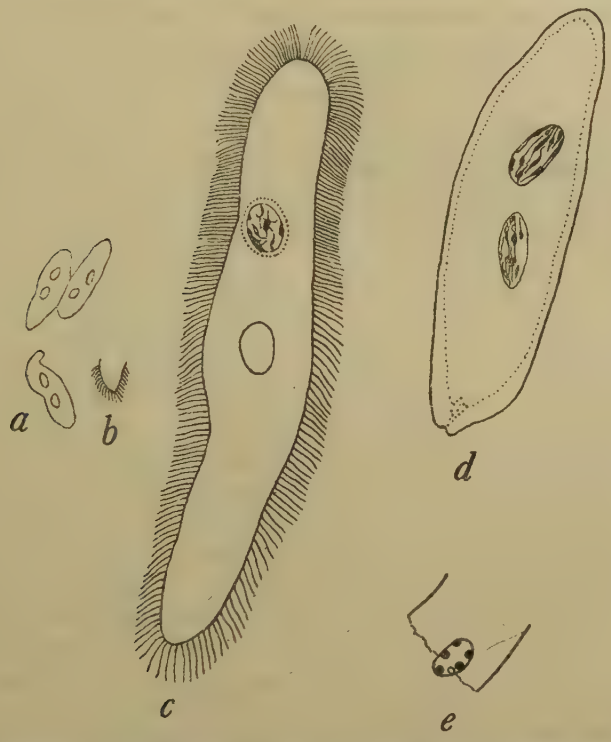

FIGURE 31.—*Protoopalina stejnegeri* Metcalf: *a*, Three individuals, × 50; *b*, posterior end of an individual slightly more magnified; *c* and *d*, × 450; *e*, × 400. (From Metcalf, 1928a.)

(small); length of nucleus 14 (large), 13 (medium), 7 (small); width of nucleus 9 (large), 8 (medium), 6 (small); cilia length 10–12; nucleoli 6.

PROTOOPALINA TRANSVAALENSIS Fantham

FIGURE 32

Host: *Bufo regularis* Reuss, from Johannesburg, South Africa.

Measurements of ordinary individuals, in microns: Body length 318–506, width 65–125. A young individual measured 165μ long and 53μ wide, a precystic individual measuring 96μ long and 28μ wide, a binucleate individual just hatched from the cyst measuring 46μ long and 18μ wide.

FIGURE 32.—*Protoopalina transvaalensis* Fantham, × 160. (After Fantham.)

PROTOOPALINA XAMACHANA, new species

FIGURE 33

Type: U.S.N.M. No. 22625.

Host: *Eleutherodactylus luteolus* (Gosse), a leptodactylid, from Jamaica, West Indies. Two preserved specimens of this host were examined, both collected by W. Harris, August 18, 1905. One showed no infection; the other, 29 mm. long, was abundantly infected. Two elongated nuclei, often dumbbell-shaped, with 4 nucleoli in each end, are usually found. The yellow hosts along with their eggs were found by Harris in the water in the cups at the bases of *Bromelia* leaves growing as epiphytes on trees on Mount Diabolo. It is interesting that these "aquatic" tadpoles in *Bromelia* leaf cups are infected like other aquatic tadpoles that live in much larger pools.

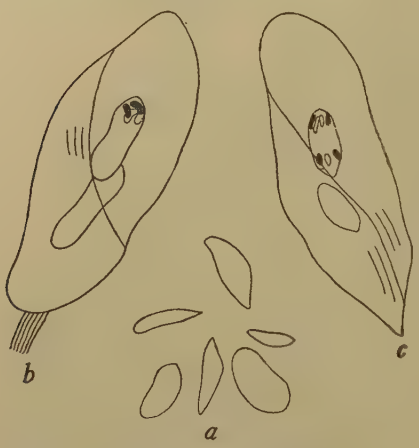

FIGURE 33.—*Protoopalina xamachana*, new species: *a*, × 124; *b* and *c*, × 505.

Measurements, in microns: Body 90 by 40, 90 by 30, 52 by 14; nucleus (elliptical, in anaphase) 18.1 by 7.1, nucleus (dumbbell-shaped, early telophase) 27 by 6.1; cilia length 10; cilia line interval 2; nucleoli 4. A few of the animals show a slight posterior point. The character of the nuclei places this species in group V.

PROTOOPALINA XENOPODOS Metcalf

I described this species from *Xenopus calcaratus* Buchholz and Peters, from the Belgian Congo. Fantham describes what he regards as the same species from *X. laevis* from Johannesburg, South Africa.

Fantham gives measurements, in microns, as follows: Body length 82–144, width 14–27; one ciliate 156 long and 31 wide; tailed gamete from tadpole 44.2 by 5.8. Nucleoli 4 (Fantham says 8 but shows 4 at each end of the dividing nucleus).

PROTOOPALINA YUNNANENSIS, new species

FIGURE 34

Type: U.S.N.M. No. 22626.

Host: *Bombina maxima* (Boulenger), from the Province of Yunnan, southwestern China, at the eastern end of the Himalaya highlands.

In the only specimen of this bell toad that I had for examination (U.S.N.M. No. 86068) four distorted Protoopalinas were found. Figure 34, *a*, shows the least distorted specimen, and figure 34, *b* and *c*, show single nuclei from pairs in two others. The nucleoli are fragmented in each case.

Measurements, in microns: Body about 230 by 46; nucleus (the longer) 18 by 10.

This species differs from *P. luzonensis* in having the nuclei near together in the center of the body instead of far apart (one far forward, the other near the middle). They are probably distinct. This is the species referred to, but not described, in my paper on the bell toads and their opalinid parasites (Metcalf, 1928a).

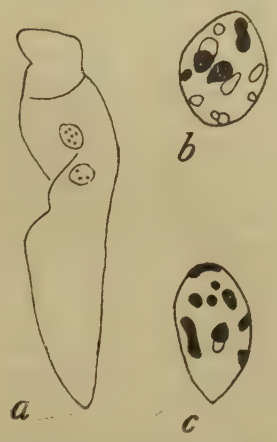

FIGURE 34.—*Protoopalina yunnanensis*, new species: *a*, ×249; *b* and *c*, the nuclei (from a second and a third individual) ×1010.

PROTOOPALINA YUNNANENSIS CHENI, new subspecies

FIGURE 35

My friend Dr. T. T. Chen sent me slides and drawings of Protoopalinas from *Bombina maxima* Boulenger collected in Yunnan, and he has kindly allowed me to include his form in this paper. It is much larger and has larger nuclei than *yunnanensis*, and in every individual seen the nuclei are united by a thread.

In each of the other species of *Bombina* studied—*igneus*, *pachypus*, and *orientalis*—there are found two species of *Protoopalina*. *Bombina igneus* and *B. pachypus*, occurring in Europe, each carry (but not in the same individual) *P. caudata* and *P. intestinalis*. *Bombina orientalis*, which is found in an extensive region centering around the base of the Korean Peninsula, carries (also in separate individual hosts) *P. macrocaudata* and *P. orientalis*. *Protoopalina caudata* has 6 nucleoli; *P. intestinalis* and *P. macrocaudata* have 8; their number is undetermined in *P. orientalis*, *P. yunnanensis*, and the form *cheni*.

Protoopalina yunnanensis and *P. cheni* more nearly resemble each other than do either of the other pairs of Protoopalinas in a species of *Bombina*, and we can indicate this by classing, say, *cheni* as a

subspecies of *yunnanensis*. It would, however, seem well to know the number of at least the nucleoli in the two Protoopalinas from *Bombina maxima* before deciding more than tentatively as to specific or subspecific divergence between them. If the numbers of the

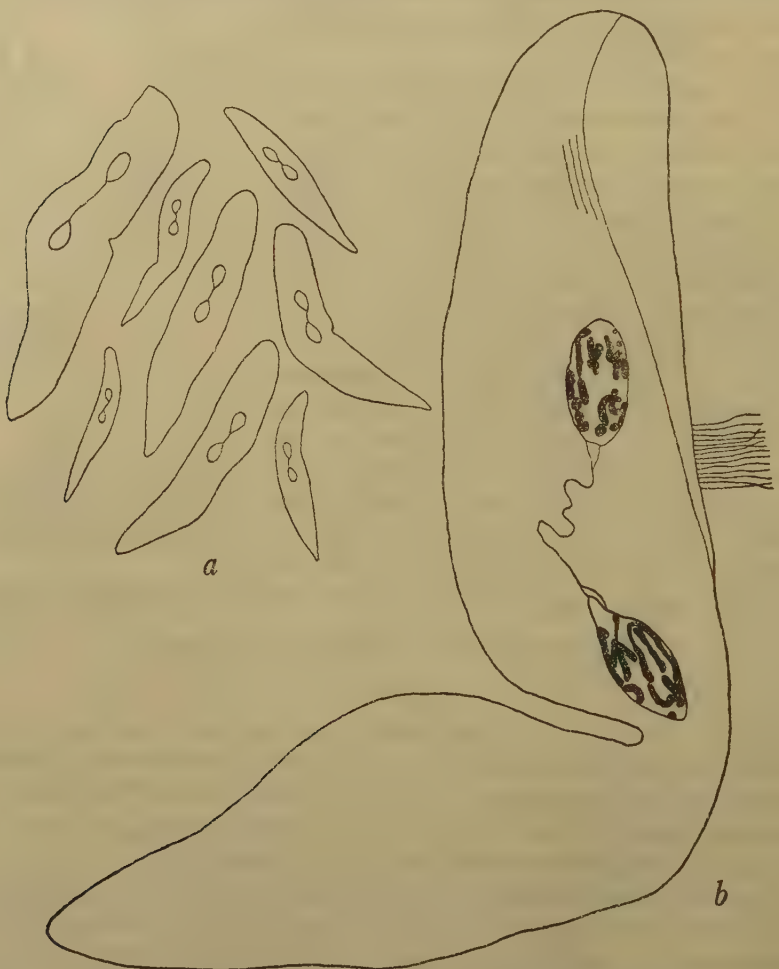

FIGURE 35.—*Protoopalina yunnanensis cheni*, new subspecies: *a*, A group of individuals from one infection, × 133; *b*, an individual × 482.

nucleoli are different in the two forms it would seem natural to class them as separate species. Among the specimens of the form Dr. Chen has sent me are none that show mitosis, and so nucleolus number has not been determined.

Bombina maxima occurs in the probable ancestral home of the Discoglossidae (see Stejneger, 1905), and its Protoopalinas may well be the most archaic of the species infecting the Bombinas. The most probable view of the origin of the Bombinas and their Protoopalinas

seems to be that *B. maxima* is the most ancient of the four hosts and that its two closely similar Protoopalinas show an early condition of evolutionary divergence. In *B. orientalis*, during its wandering to its present home near the base of the Korean Peninsula and during its subsequent residence there, the two Protoopalinas, probably already present, diverged still farther to give the now very distinct species *P. macrocaudata* and *P. orientalis*. Two Bombinas, or perhaps their common ancestor, wandered north from southwestern China and turned westward to reach Europe. During this extensive period of migration and of residence in the west, the species evolved into two, one, *B. igneus*, now living in the low country, the other, *B. pachypus*, living among the hills, although their habitats overlap. *B. igneus* breeds usually in larger pools of still water or in sluggish streams, while *B. pachypus* is more likely to lay its eggs in small, perhaps transient, pools, even in puddles in wheel ruts. Probably as a result of their overlapping habitats and the consequent at least occasional common breeding pools, the two hosts still carry the same two species of *Protoopalina*. If the altitude preferences of the two species of *Bombina* should become more sharply distinct, causing them to breed always in separate pools, *B. igneus* only at low altitudes, *B. pachypus* only at higher altitudes, opportunity would be given for evolution to develop in each host its own distinctive two species of parasites, as has occurred in *B. orientalis*.

Measurements, in microns, of *Protoopalina yunnanensis cheni:*

Measurement	*a*	*b*	*c*
Length of body	400	450	250
Width of body	83	90	------
Length of daughter nuclus	52	43	------
Width of daughter nucleus	20	21	------
Length of cilia	24	------	------
Interval between lines of cilia, anterior	1. 3	------	------

PROTOOPALINA LIMNOCHARIS Nie

FIGURE 36

Host: *Rana limnocharis* Gravenhorst, from Nanking, China.

Body is rather flattened and about 10 times as long as broad. The greatest width is at a region a little anterior to the middle of the body, and from there the body tapers very gradually toward the ends.

The two pear-shaped nuclei are connected by a long thread. The anterior nucleus is situated at about the anterior sixth, while the posterior one lies a little anterior to the middle part of the body. Each nucleus contains about 10 nucleoli of different size and shape and irregularly disposed.

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The layer of ectosarc is rather thick and consists of very large and prominent alveoles. The endospherules in the endosarc are large, being ellipsoidal, rounded, or pear-shaped.

Measurements for a number of specimens, in microns: Length of body 238–428.4, width 22.8–36.1; length of nucleus 22.8–26.6, width

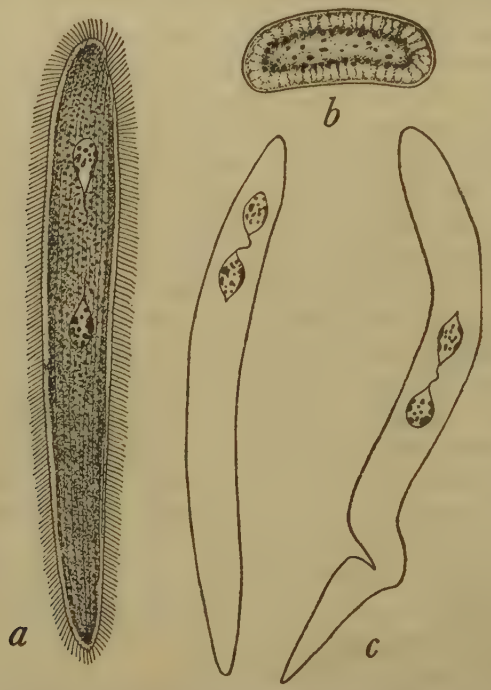

FIGURE 36.—*Protoopalina limnocharis: a*, Structure of the animal, × ca. 250; *b*, cross section, posterior to the midregion, × ca. 500; *c*, two young animals soon after binary fission, × ca. 250. (After Nie.)

9.5–13.3. The measurements of some of the endospherules show the average length to be 2.7 μ, and the average width 0.9 μ. The measurement of the cilia line interval of one animal was 1.9 μ on the anterior end and 3.4 μ on the posterior end.

This species seems to be closely related to *Protoopalina filiformis* Metcalf (1923a). It differs from the latter mainly in number of nucleoli (6 in *P. filiformis*). The body is more flattened and the endospherules are much larger as compared with *P. filiformis*.

PROTOOPALINA PINGI Nie

FIGURE 37

Host: *Rana plancyi* Lataste, from Nanking, China.

This species is relatively small. The body is somewhat spindle-shaped, the posterior end being narrow or sharply pointed while the anterior end is somewhat narrow and rounded. The animal is usually

bent to one side at the anterior end. The layer of ectosarc is fairly thick. The endosarc in the axial region of the body contains numerous endospherules, and 4 to 6 (usually 4) ellipsoidal or rounded nuclei. The number of the nucleoli, as seen in midanaphase, are distinctly 6.

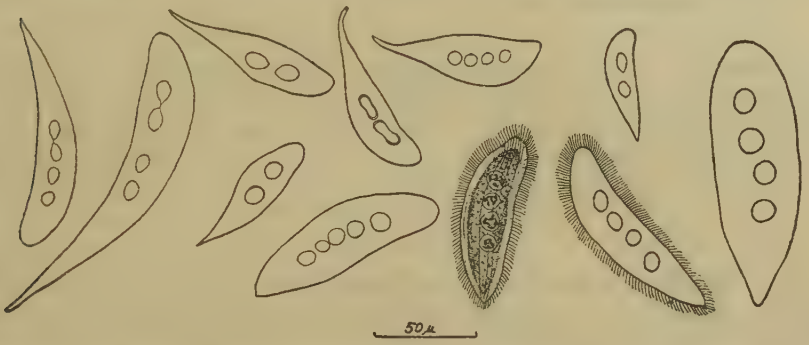

FIGURE 37.—*Protoopalina pingi* Nie: A group of individuals showing the range of size and shape. (After Nie.)

In a number of animals measured length of the body varies from 55 to 160μ, width of the body from 12.5 to 26μ, diameter of the nucleus from 10 by 5μ to 12.5 by 7.5μ.

Genus ZELLERIELLA Metcalf

ZELLERIELLA BRASILIENSIS (Pinto)

FIGURE 38

Host: *Crossodactylus gaudichaudii* Duméril and Bibron, from Rio de Janeiro, Brazil, one specimen, 16 mm. long, uninfected, and a

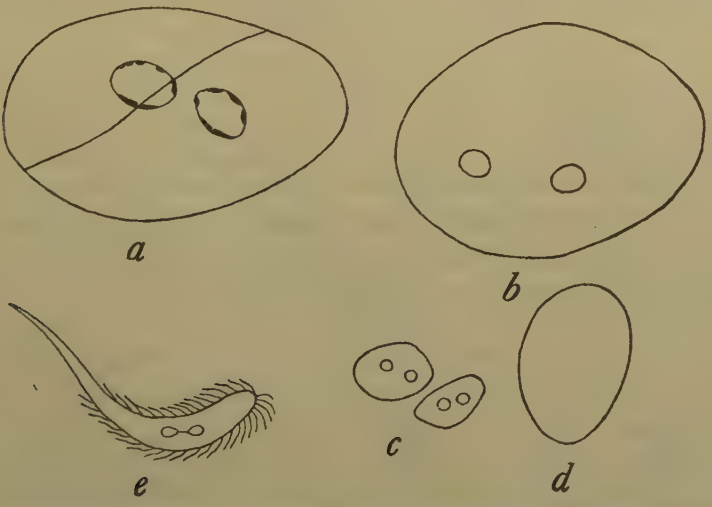

FIGURE 38.—*Zelleriella brasiliensis* (Pinto) from *Crossodactylus gaudichaudii*: *a*, × 460; *b*, × 249; *c*, × 117; *d*, × ?; *e*, × 1010.

second, 31 mm. long, very abundantly infected; also a tadpole, 55 mm. long, in which were found some *Protoopalina*-like larvae.

Measurements, in microns:

Measurement	*a*	*b*	*c*	*d*	*e*	*f*	*g*
Body length	165	100	86	50	40	72	20
Body width	128	65	61	25	14	100	84
Nucleus length	18. 7	19	15	5	2	12	9. 1
Nucleus width	15	13	13	4	1. 2	-----	-----
Cilia line interval	-----	-----	-----	-----	-----	-----	1. 8

ZELLERIELLA BRASILIENSIS (Pinto) (?)

FIGURE 39

In a tadpole of *Leptodactylus ocellatus* (Linnaeus) from Manguinhos, Rio de Janeiro, Brazil, were two sorts of opalinids, one *Zelleriella*

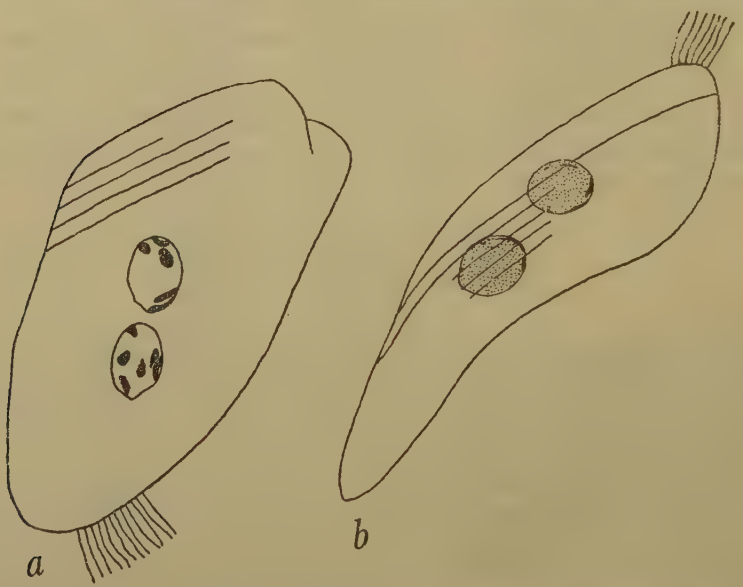

FIGURE 39.—*Zelleriella brasiliensis* (Pinto) (?) from a tadpole of *Leptodactylus ocellatus*, × 820.

(fig. 39), the other *Cepedea*. The tadpole measured (in mm.): Total length 59, length of body 18, length of hind legs 12; no forelegs visible.

The Zelleriellas measured, in microns:

Measurement	*a*	*b*
Body length	82	90
Body width	39	22. 5
Nucleus length	12. 2	12
Nucleus width	8. 3	9
Cilia length	9	9
Interval between lines of cilia	2	2

Nucleoli seemingly 6, but they were not observed in anaphases of mitosis, where they are most easily and reliably counted. The second individual shown in the figure (fig. 39, *b*) seems almost a *Protoopalina* and represents, doubtless, a *Protoopalina* stage in the development. The species is probably *brasiliensis*, the usual one found in *Leptodactylus ocellatus*, but study of more material would be necessary to determine. The *Cepedea* present in the same tadpole is described on page 512. There are in the infection a number of individuals that are abnormal, as indicated by one of their two nuclei staining uniformly dark with Delafield's haematoxylin (fig. 39, *b*).

ZELLERIELLA URUGUAYENSIS, new species

FIGURE 40

Type: U.S.N.M. No. 22627.

Host: *Bufo arenarum* Hensel. Five specimens from Montevideo were infected; 16 from the same locality carried a huge *Zelleriella* up to four times as large (linear dimensions) as Pinto's *Z. brasiliensis*, with which it might carelessly be confused.

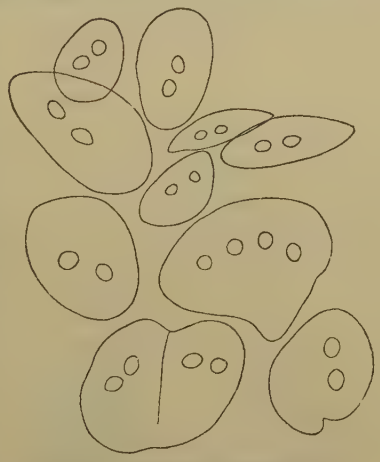

FIGURE 40.—*Zelleriella uruguayensis*, new species, from *Bufo arenarum*, × 59.

The largest specimens of Pinto's species (I have studied his slides) are much smaller than the smallest from *B. arenarum*, and the nuclei are situated farther back in the body. The largest individuals of *Z. magna*, from Venezuela, in *Leptodactylus typhonius* (Daudin) are as large as the smallest specimens in *B. arenarum*, but the shape is very different. The largest *Z. opisthocarya*, from Nicaragua, in *B. coniferus* Cope are as large as medium-sized specimens from *B. arenarum* and they agree in shape, and the position of the nuclei is usually well back on the body, but the nuclei are but half as large as in the Montevideo species. This is clearly distinct.

Measurements, in microns:

Measurement	*Dividing, 4 nuclei*	*Large*	*Medium*	*Small*
Body length	338	441	327	245
Body width	401	247	195	140
Nucleus length	55	65	44	25. 7

The nucleoli are probably 6 in number.

Two individuals were found broader than long and with four nuclei in a line across the much widened body. I interpret this as due to the delay of one longitudinal division. A much elongated individual with 10 circular nuclei I interpret as due to repeated nuclear division without fission. The direction of the rows of cilia in this specimen is nearly transverse to the greatest length of the body. The condition is still more abnormal than that shown in the 4-nucleated forms, more than two morphologically longitudinal lesions having been omitted and the "width" having become greatly overdeveloped. The host of these Infusoria had been kept in captivity for about 10 days before its parasites were studied.

BALANTIDIUM URUGUAYENSIS QUADRATA, new form

Figure 42

Type: U.S.N.M. No. 22628.

Host: *Bufo dorbignyi* Duméril and Bibron.

In this host from Rio de Janeiro, and perhaps also from Montevideo (records confused), are many infections containing individuals

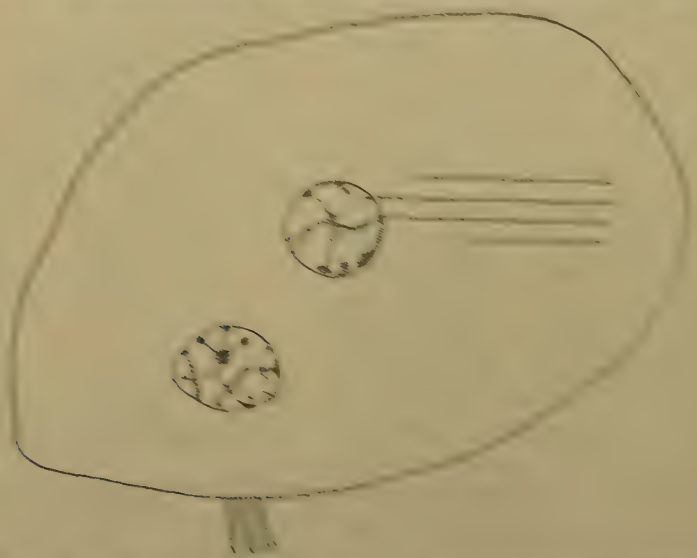

FIGURE 42.—*Balantidium uruguayense quadrata*, new form, from a tadpole of *Bufo dorbignyi*. × 50.

shaped like and as large as small *B. uruguayense* and they have as large nuclei; among these are other individuals having a more or less truncate posterior end. Comparison with undeveloped *B. minutum*, to be described, from tadpoles of *Bufo crucifer* suggests that the broadly truncate condition is due to what is really an undeveloped point or rudimentary tail at one posterior angle, the other being rounded. In some individuals the posterior end appears split, the nascent point

being demarcated by a furrow at its base from the rest of the posterior end of the body. This is an intermediate condition between the "species" *uruguayensis* and *antunesi*. I am describing it as a form of *Z. uruguayensis*.

Measurements, in microns:

Measurement	*a*	*b*	*c*	*d*	*e*
Body length	276	268	223. 8	------	-----
Body width	184	214	156	------	-----
Nucleus length	34. 4	36	25. 2	57. 2	35. 2
Nucleus width	26. 8	24	25. 2	21. 2	30
Cilia line interval, posterior	3	-----	-------	------	-----
Cilia line interval, anterior	2. 5	-----	-------	------	-----

Nucleoli 6.

The form *quadrata*, with the same general measurements, is found also in *Bufo crucifer*.

ZELLERIELLA ANTUNESI Pessôa

FIGURES 42, 43

Hosts: *Leptodactylus ocellatus* (Linnaeus), *Bufo crucifer* Wied, *B. dorbignyi* Duméril and Bibron, and *B. arenarum* (Hensel).

In these four hosts at Rio de Janeiro and at Montevideo there are Zelleriellas that it is difficult to distinguish, for after study of many infections one realizes that they seem to grade into one another. One form, *Z. antunesi*, is astonishing. It is usually quite distinct, but in a few infections its most distinctive character, the remarkable tail, is but little developed. When the tail is in the usual condition, the body of the animal is almost double, consisting of a lower portion without tail and a second upper portion fused in front with the lower, but becoming more and more elevated behind, until at the back of the body it forms a high, narrow ridge, which is drawn out to a cylindrical, pointed tail projecting upward and backward to a distance almost equal to half the length of the body. Study of individuals in which the tail is developed to varying degrees, especially those infrequent individuals in which it is almost rudimentary, shows that in its fundamental morphology the tail is the posterior point that occurs in many species of all four genera of opalinids. It will be discussed later.

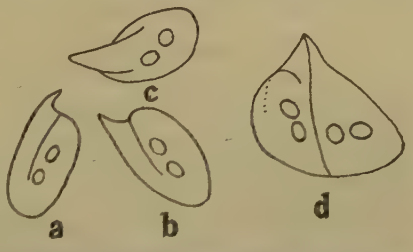

FIGURE 42.—*Zelleriella antunesi* Pessôa: a–c, From *Bufo crucifer*; d, from *Leptodactylus ocellatus*. All × 117.

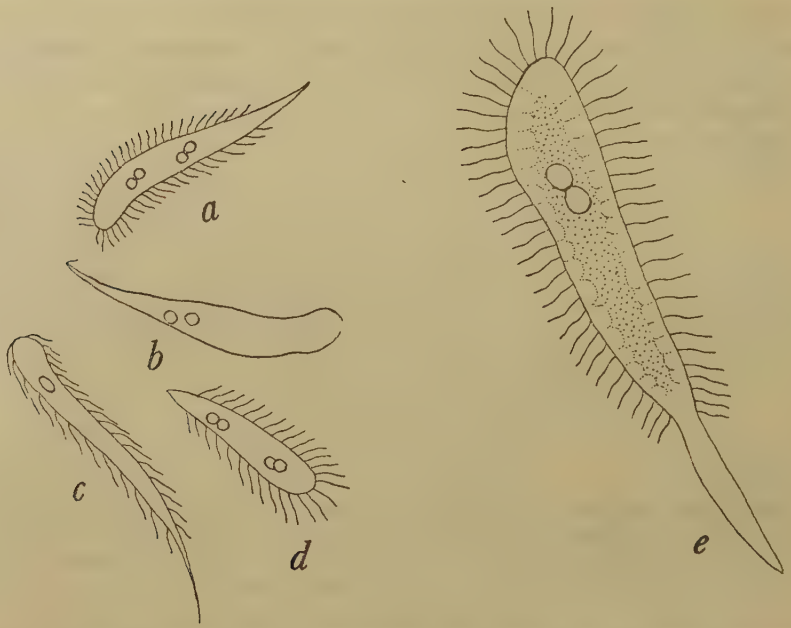

FIGURE 43.—*Zelleriella antunesi* Pessôa (?) from tadpoles of *Bufo crucifer:* Free-hand drawings from life or from nonpermanent specimens treated with acetic acid or with acetocarmine. Magnifications not recorded. All are in *Protoopalina*-larval stages.

Measurements, in microns, of 4 individuals—*a*, *b*, and *c* from *Bufo crucifer*, *d* from *Leptodactylus ocellatus:*

Measurement	*a*	*b*	*c* (in fission)	*d*
Length of main body	89	121	----------	100
Length to tip of tail	146	149	175	125
Width of body	68	70	23	60
Length of nucleus	22. 9	23	23	17
Width of nucleus	12. 8	13. 9	15	12. 3

ZELLERIELLA ANTUNESI QUADRATA, new forma

Type: U.S.N.M. No. 22629.

Host: *Bufo crucifer* Wied, from Rio de Janeiro, Brazil.

Of 24 individuals of this toad, 12 were uninfected with opalinids, 5 bore abundant *Z. antunesi*, 2 showed *Z. uruguayensis*, and 5 bore intermediate forms either with slight tails or a peculiar angular contour, not protuberant, where a tail might have developed. These last might almost be called forma *quadrata*. Their small nuclei differentiate them from the species *uruguayensis*. In a small pond,

with some adult toads, were many tadpoles; of those examined several bore interesting small Zelleriellas, with tails developed to different degrees, but none were found of fully adult shape. In one smaller tadpole were *Zelleriella* larvae in the *Protoopalina* stage and other cells that perhaps were stages in the development of male gametes.

Figure 43 shows free-hand sketches from living parasites or nonpermanent preparations from tadpoles of *B. crucifer*. They resemble Protoopalinas of what I have considered the most archaic subgeneric group. They have elongated posterior ends, slender, unciliated, and sharp-pointed. One, not drawn, shows two nuclei still united by a thread after division. Another shows a single nucleus with several axial excretory vacuoles in front of and behind it. These individuals may represent *Zelleriella* larvae in a *Protoopalina* stage or they may be stages in the development of male gametes. These forms from the tadpole host were studied with a magnification of 1,010 diameters after staining with acetocarmine and were not kept.

Of 28 adult specimens of *Leptodactylus ocellatus* from Rio de Janeiro, 15 bore no opalinids, 13 were infected with large or small Zelleriellas. Fourteen of these frogs had been in captivity for six weeks or more, and on this account the opalinids may have disappeared from some of them. Some of the parasites were *Z. uruguayensis*, some *Z. brasiliensis*, a few were *Z. antunesi*, and some were intermediate, such as I am calling forma *quadrata*. In one individual typical *Z. brasiliensis* and *Z. antunesi* were present, their nuclei being large in *antunesi* and small in *brasiliensis*. This is one of the few instances in which I have found in one individual host what seem to be two species of opalinids.

The conditions of parasitism in *Leptodactylus ocellatus*, *Bufo dorbignyi*, *B. crucifer*, and *B. arenarum*, both in Brazil and in Uruguay, are very puzzling. There are large Zelleriellas with large nuclei (*"uruguayensis"*), small Zelleriellas with nuclei of half the relative size (*"brasiliensis"*), tailed forms (*"antunesi"*) with nuclei of the relative size found in *"uruguayensis,"* and posteriorly truncate forms (*"quadrata"*) with nuclei relatively large. Through *quadrata* forms *antunesi* seems to grade into *Z. uruguayensis* but not into *Z. brasiliensis*.

ZELLERIELLA DUBIA, new species

FIGURE 44

Type: U.S.N.M. No. 22630.

Host: *Eupemphix nana* Boulenger. Three specimens from Angra dos Reis, State of Rio de Janeiro, Brazil, showed no opalinids; three were heavily infected.

Measurements, in microns:

Measurement	*a*	*b* (dividing)	*c*
Length of body	180	124	152
Width of body	130	148	100
Nucleus length	------	25	20
Nucleus width	------	10	18. 5
Cilia line interval	------	----------	3. 9

Many of these Zelleriellas show numerous cytoplasmic parasites, the amoebae (Stabler and Chen, 1936), which were not studied. This species is thinner behind than in the front part of the body, but

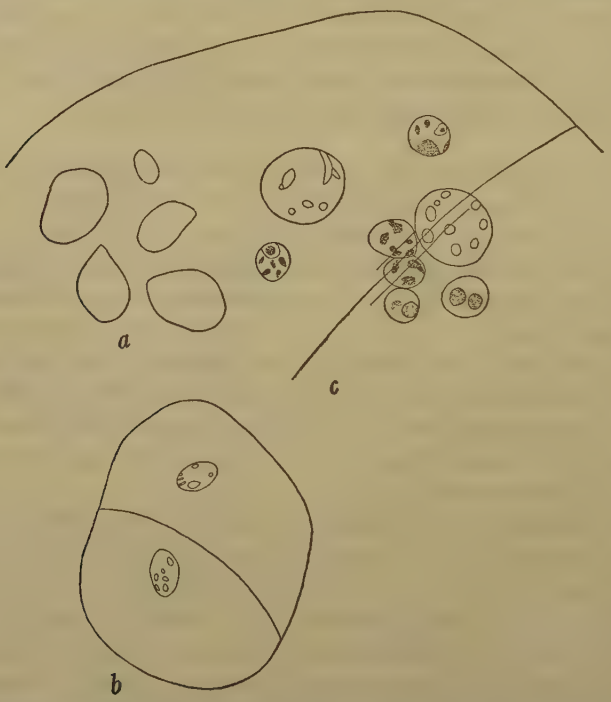

FIGURE 44.—*Zelleriella dubia*, new species: *a*, × 59; *b*, × 230; *c*, × 505. In *c* the six smaller bodies are parasites. Further work of Stabler and Chen (1936) shows that they are amoebae.

the posterior edge is not quite so conspicuously thin as in some forms of the genus *Opalina*, e. g., *O. ranarum* form *truncata* (Metcalf, 1923a).

This large *Zelleriella* is of different form from *Z. magna*. It differs from the *opisthocarya* group in the more anterior position of its nuclei. None of the specimens shows so definite a posterior point as does *Z. patagoniensis*. It is much larger and has relatively much larger nuclei than *Z. brasiliensis*. Studying whole infections, shape, size, and measurements, I am impressed that it is a distinct species, if

indeed there be distinct species in this exasperating genus that refuses to play the Linnaean game, but it is difficult to give a diagnostic description. Yet, on the basis of the strong impression from the study of whole infections of this and other Zelleriellas it resembles, I am giving it a distinctive name, pending more detailed study of further material from this species of host.

ZELLERIELLA OVONUCLEATA, new species

FIGURE 45

Type: U.S.N.M. No. 22631.

Host: *Leptodactylus pentadactylus* (Laurenti). One specimen from Bello Horizonte, Brazil, 135 mm. long, was uninfected. A rectum of another specimen from the same locality, given me by Dr. Lauro Travassos, showed very few Zelleriellas.

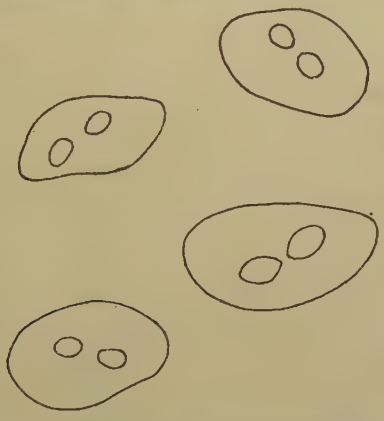

FIGURE 45.—*Zelleriella ovonucleata*, new species, × 249.

Measurements, in microns:

Measurement	Large	Small
Length of body	104	83
Width of body	58	42
Nucleus length	23	15. 7
Nucleus width	18	9

In size and shape of body and of nuclei, and in relative size of body and nuclei, this form agrees with a *Zelleriella* from *Bufo sternostignotus* Keferstein (see Metcalf, 1923a). The nuclei of the latter form were never found so elongated as in the form from *Leptodactylus pentadactylus*, but the difference is slight. I am naming the species *Z. ovonucleata* and am naming the form formerly mentioned tentatively as *Z.* [of *Bufo sternostignotus*] as subspecies *bufonis* of the species *ovonucleata*.

ZELLERIELLA [of Eleutherodactylus miliaris]

FIGURE 46

Host: *Eleutherodactylus miliaris* (Spix), from Angra dos Reis, State of Rio de Janeiro, Brazil.

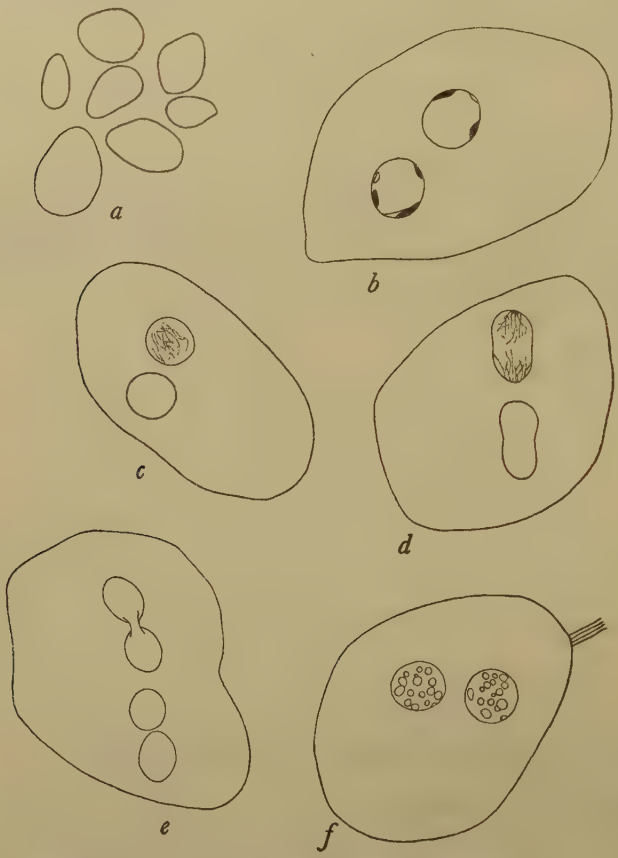

FIGURE 46.—*Zelleriella* [of *Eleutherodactylus miliaris*]: First group of figures (*a*) × 146; all others × 673.

Of three specimens, about 55 mm. long, one was very abundantly infected with a rather small *Zelleriella*. Measurements, in microns:

Measurement	*a*	*b*	*c*	*d*
Length of body	90	91	76	11. 7
Width of body	70	55	89	53
Nucleus length	------	15. 1	19	11. 7
Nucleus width	------	13. 1	13. 8	8. 7
Length of cilia	------	9	------	------

Nucleolus number undetermined. In many nuclei they are not seen at all, the nuclei being parasitized. In shape of body and large size of nuclei compared with the size of the body, this form is close to

one I have referred to, without definitely naming, as *Z.* [*trinitatis*] (Metcalf, 1923a) and to *Z. hylaxena* Metcalf (op. cit.), but the dividing nuclei differ from those of the latter species. It should not be named without more detailed study of nonparasitized material.

ZELLERIELLA [TRINITATIS] Metcalf

FIGURE 47

Host: *Elosia lateristrigata* Baumann,[3] one specimen, from Angra dos Reis, State of Rio de Janeiro, Brazil.

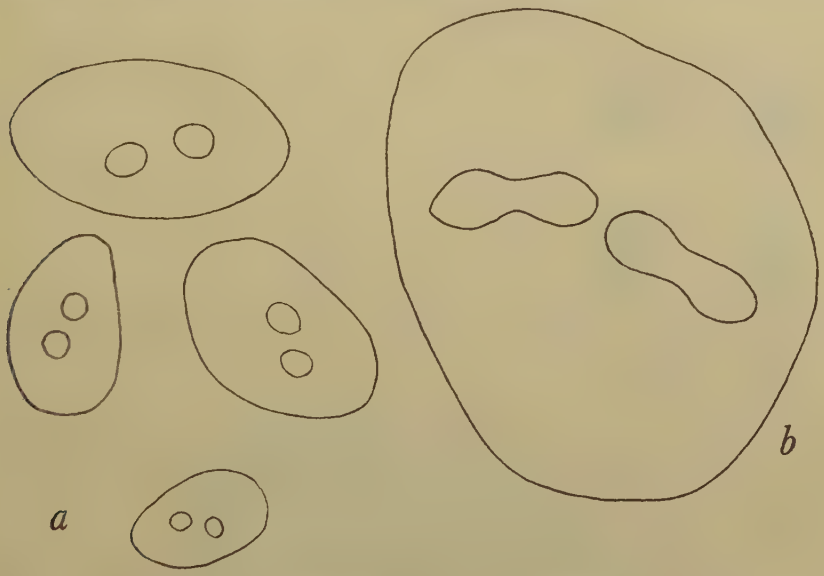

FIGURE 47.—*Zelleriella* [*trinitatis*] Metcalf from *Elosia lateristrigata*: *a*, × 249; *b*, × 460.

Measurements, in microns:

Measurement	Large	Large (dividing)	Small
Length of body	147	130	80
Width of body	86	150	48
Length of nucleus	24. 5	50	12. 5
Width of nucleus	17. 2	16. 9	10

This form resembles *Z.* [*trinitatis*] Metcalf (1923a) from *Phyllobates trinitatis* Garman, from Venezuela, but the individuals from *Elosia* were larger. Detailed cytological study of infections from both hosts, which my material has not allowed, might well show diagnostic differences, so I am only provisionally assigning the present forms to the Venezuelan species, which itself was only provisional.

[3] Possibly a wrong identification.

ZELLERIELLA PISCICOLA Da Cunha and Penido

FIGURE 48

Da Cunha and Penido (1926) reported a *Zelleriella* in a "catfish" from the Paraguay River.

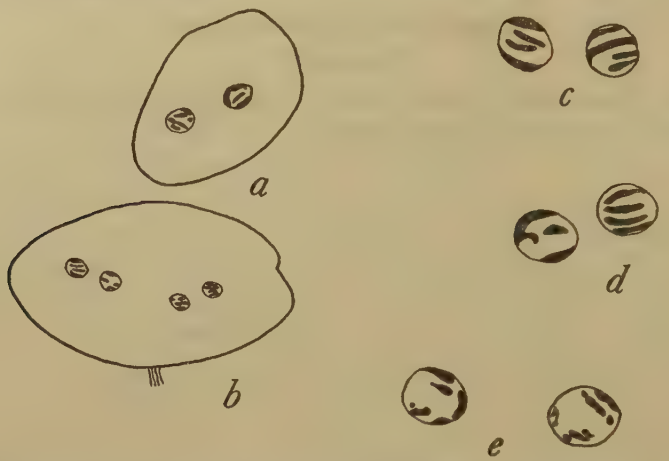

FIGURE 48.—*Zelleriella piscicola* Da Cunha and Penido: *a*, An ordinary individual; *b*, an individual in division; *c* and *d*, pairs of nuclei after division; *e*, a pair of resting nuclei. Magnification not indicated. (After Da Cunha and Penido.)

Measurements, in microns: Body length 70–110; body width 50–60; cilia 10–12, longer in front, shorter behind. Nucleoli, 4 in number, show individual constant differences from each other during mitosis, but there are not two of each kind. (See the discussion of *Protoopalina caccosterni*, p. 469, for interpretation.)

ZELLERELLA ORIENTALIS Nie

FIGURE 49

Host: *Microhyla ornata* Boulenger, from Nanking, China.

The outline of the body is roughly leaflike. It is broadest at the anterior, which is slightly curved and somewhat obliquely truncated. The posterior portion is the narrowest and terminates abruptly to a minute sharp point. The cilia are arranged in many longitudinal or slightly oblique rows. The cilia vary in length, those of the anterior end are 14.1μ, while posteriorly they average 11.7μ. The two large nuclei are ellipsoidal, one being located at the anterior half and the other near the middle or at the posterior portion of the body. Each nucleus contains eight (?) nucleoli in midanaphase. The chromosomes are very distinct and more numerous than the nucleoli. The endospherules are either rounded or dumbbell-shaped.

Measurements, in microns: In a number of individuals length of the body varies from 87.5 to 120, width of body 45.0 to 70, thickness of body 19.6 to 32.2; diameter of nucleus from 12.5 by 9.6 to 16.0. The measurement of the cilia line interval of one animal was 3.1 at the anterior end, 4.5 in the middle portion, and 6.3 at the posterior end.

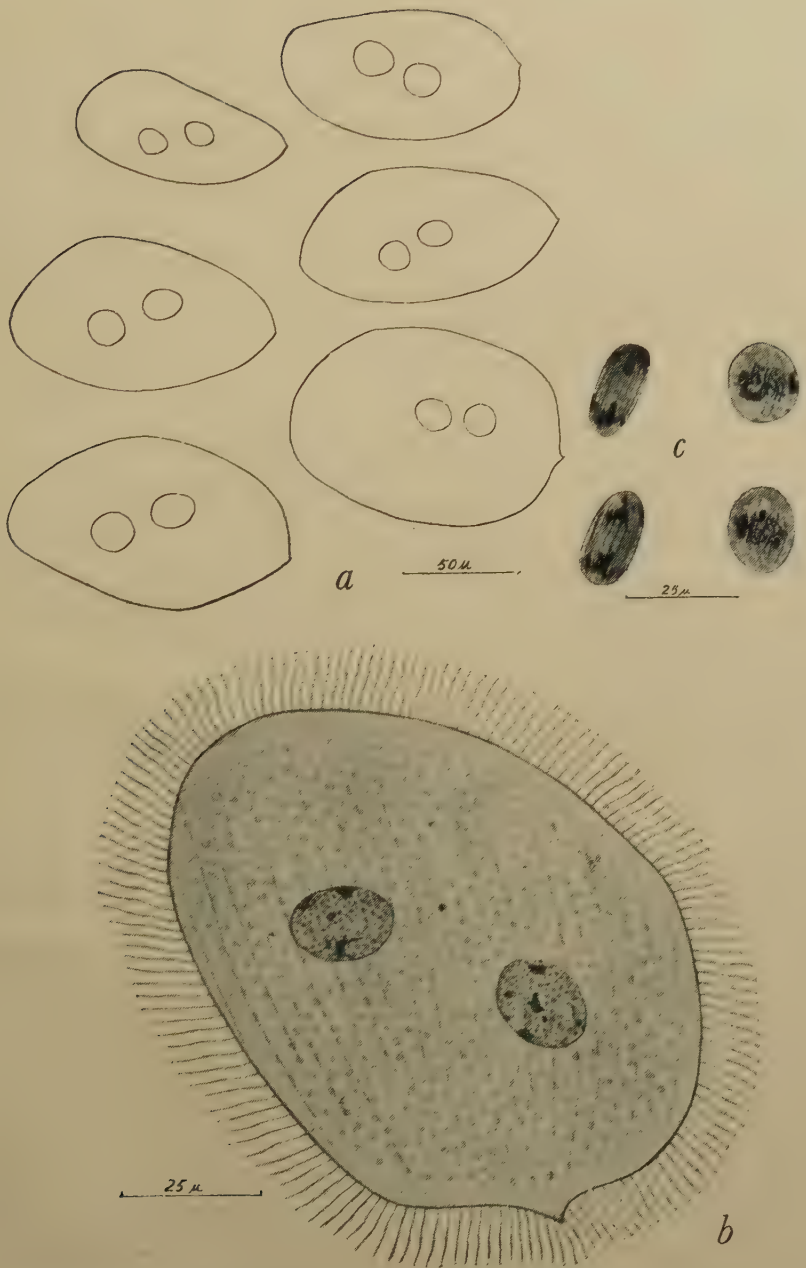

FIGURE 49.—*Zelleriella orientalis* Nie from *Microhyla ornata*: *a*, A group of animals showing the range of size and form; *b*, showing the structure of the animal; *c*, two pairs of dividing nuclei. (After Nie.)

ZELLERIELLA CORNUCOPIA Carini

FIGURE 50

Host: *Leptodactylus ocellatus* (Linnaeus), from Brazil.

The body is flattened and curved. The anterior end is much wider than the rest of the body. The outline of the animals appears to be

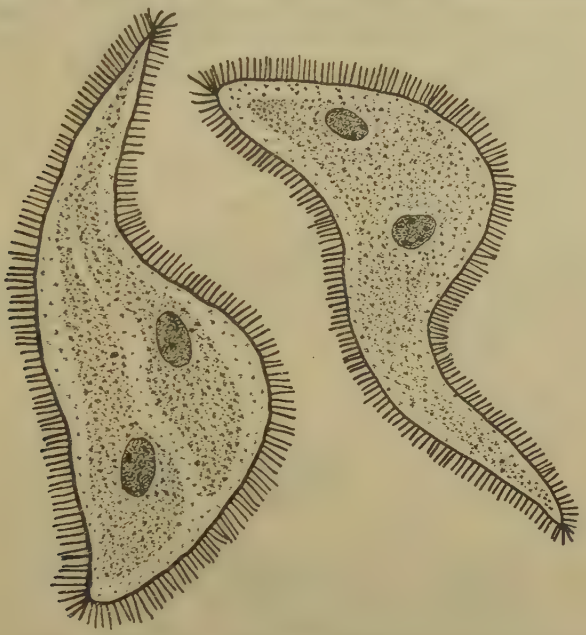

FIGURE 50.—*Zelleriella cornucopia* Carini from *Leptodactylus ocellatus*, × ca. 380. (After Carini.)

trumpet-shaped. The larger specimens measure 180–220μ in length; in these the anterior end is 75–100μ in width. The two spherical nuclei measure 20–22μ in diameter.

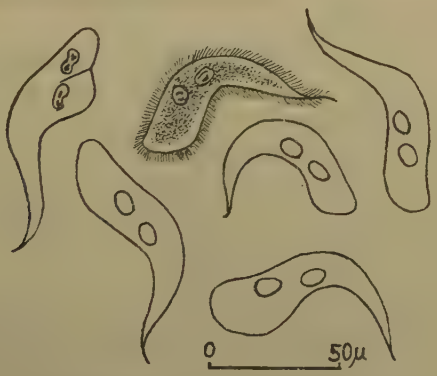

FIGURE 51.—*Zelleriella corniola* Carini from *Leptodactylus ocellatus*. (After Carini.)

ZELLERIELLA CORNIOLA Carini

FIGURE 51

Host: *Leptodactylus ocellatus* (Linnaeus), from Brazil.

The body is slightly flattened, long, and curved. This species measures 70–90μ in length, and the anterior end is the region of greatest width. From there, the animal gradually decreases in width toward the posterior end, which is pointed, and in some specimens twisted. Two nuclei, 8–10μ in diameter, are located about 5μ from each other in the anterior half of the body.

ZELLERIELLA FALCATA Carini

FIGURE 52

Host: *Engystoma ovale* Schneider, from Brazil.

This species of *Zelleriella* found in São Paulo is very much flattened and often presents a certain degree of twisting. It has a rather

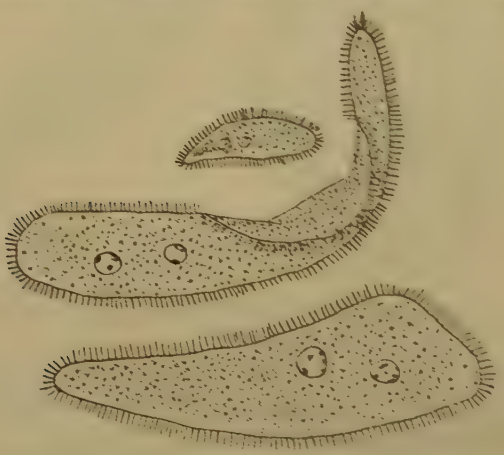

FIGURE 52.—*Zelleriella falcata* Carini from *Engystoma ovale*. No magnification given. (After Carini.)

variable form, sometimes spindle-shaped, sometimes cone-shaped; however, the form that has been observed most frequently and that appears to be the most typical is that of a comma, more or less elongated. The anterior end is large and obtuse, the posterior end slender.

The size is highly variable. The small forms are often fusiform and measure nearly 50–100μ in length and 20–30μ in width. The well-developed individuals, with the characteristic form of a comma, measure 200–300μ in length and 40–80μ in width.

The two spherical nuclei, about 15–17μ in diameter, are found at the anterior part of the animal. Each nucleus has nucleolar substance in the form of small irregular blocks. The two nuclei are situated obliquely to the axis of the body. The distance between the two nuclei is about 20–25μ.

ZELLERIELLA FOLIACEA Carini

FIGURE 53

Host: *Leptodactylus ocellatus* (Linnaeus), from Brazil.

This species of *Zelleriella* has an irregular, round outline. The body is very thin, having the appearance of a leaf. The body sometimes shows longitudinal folds. It resembles *Z. leptodactyli* Metcalf (1923a), which is much smaller. The majority of the animals belong-

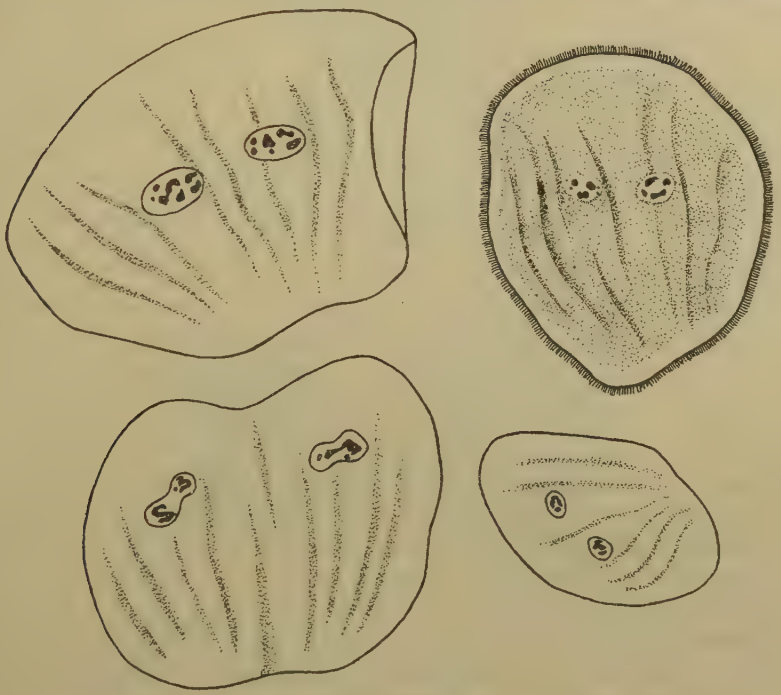

FIGURE 53.—*Zelleriella foliacea* Carini from *Leptodactylus ocellatus*. (After Carini.)

ing to *Z. foliacea* measure 200μ in diameter; some of them are over 300μ. The two spherical nuclei measure 25μ in diameter. Eight nucleoli have been observed in this species.

ZELLERIELLA TRUNCATA Carini

FIGURE 54

Hosts: *Leptodactylus ocellatus* (Linnaeus) and *L. sibilatrix* (Wied), from Brazil.

This species of *Zelleriella* is truncate in shape. These animals are found only rarely and almost always with other Zelleriellas that are regularly oval in shape. The anterior end of *Z. truncata* is rounded,

the posterior end appearing as if cut off at the tip. The protoplasm at the posterior end is less dense and highly vacuolated. These

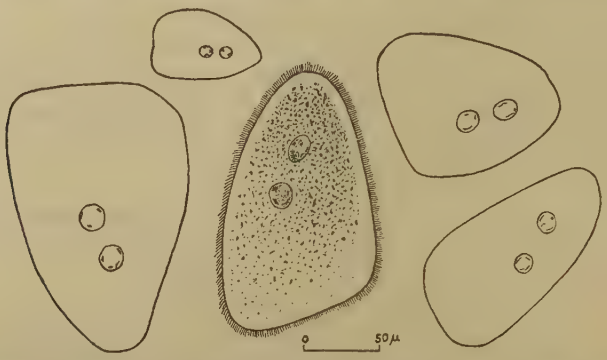

FIGURE 54.—*Zelleriella truncata* Carini from *Leptodactylus ocellatus*. (After Carini.)

animals measure 80–150μ in length and 45–65μ in width. The two nuclei located in the middle of the body are spherical, measuring 10–12μ in diameter. (A restudy of this species is desirable.)

ZELLERIELLA BOIPEVAE Carini

FIGURE 55

Host: A snake, *Ophis meremmii* (Wagler), from Brazil.

This species of *Zelleriella* was found in considerable number in the terminal portion of the intestine of the snake. These opalinids are irregularly oval in shape, the anterior part of the body being a little thinner than the posterior part. They measure 100–150μ in length and 60–90μ in width. The body is flattened and has a thickness of 25–35μ. There is a narrow zone of ectoplasm. The endoplasm is vacuolated and contains many endospherules. Two spherical nuclei,

FIGURE 55.—*Zelleriella boipevae* Carini from *Ophis meremmii*, × ca. 335. (After Carini.)

located in the middle of the body, measure 12–15μ in diameter; they are 15–20μ apart. The nucleolar substance is either in the form of a compact block or oftener in the form of irregularly distributed blocks adjacent to the nuclear membrane.

This snake feeds on anurans, and it is possible that this is merely an adventitious infection. (The description of this species is not sufficient to allow comparison with other species and determination of its affinities.)

ZELLERIELLA JAEGERI Carini

FIGURE 56

Host: A snake, *Liophis jaegeri* (Günther), from Brazil.

The body is oval, greatly flattened. This species of *Zelleriella* averages 60–73μ in length and 34–42μ in width. Two spherical or slightly oval nuclei are found in the midregion of the body, measuring 10–12μ in diameter. They are 5–8μ apart.

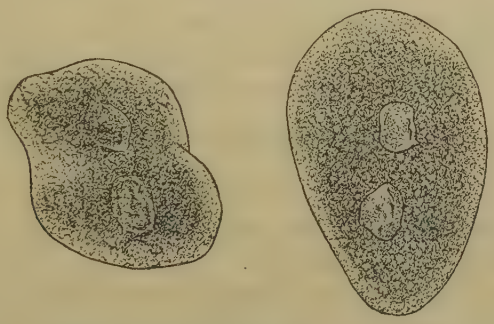

FIGURE 56.—*Zelleriella jaegeri* from *Liophis jaegeri*, × ca. 560. (After Carini.)

ZELLERIELLA ARTIGASI Unti

Host: *Bufo marinus* (Linnaeus), from Brazil.

The body is oval and flattened, measuring 60–70μ in length and 30–40μ in width. The shape of the body in the majority of the specimens is that of an egg of *Schistosoma mansoni*. Characteristic of this species is the presence of a transparent tail, which is devoid of cilia. Both nuclei are spherical, measuring on the average 9–12μ; they are 6–7μ apart. Within each nucleus one, two, or three nucleoli of different shapes are found adjacent to the nuclear membrane.

ZELLERIELLA species (?)

FIGURE 57

Host: *Elosia lateristrigata* Baumann. Of six specimens from Angra dos Reis, State of Rio de Janeiro, Brazil, two were infected.

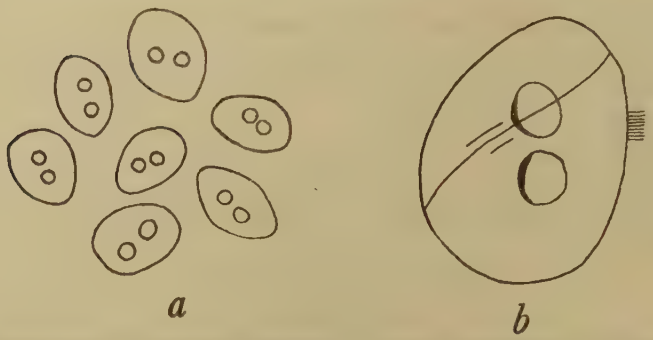

FIGURE 57.—*Zelleriella* sp. (?) from *Elosia lateristrigata: a*, A group of individuals from one infection, × 207; *b*, an individual × 750.

Genus CEPEDEA Metcalf

CEPEDEA SAHARANA Metcalf

Host: *Rana esculenta ridibunda* Pallas.

This *Cepedea* from this frog was first described from Algiers. Gourvitsch (1926) later reported it from Tashkent, Turkestan, under the name *Opalina elongata* (see Metcalf, 1927b). I have since found it in the same host from Beluchistan (U.S.N.M. No. 26194).

CEPEDEA BUERGERI SINENSIS Metcalf

FIGURE 58

Host: *Bufo gargarizans* Cantor, imported from China and given me by Dr. K. K. Chen, of Eli Lilly & Co.

Figures of these stocky specimens are given and some measurements, in microns, from the one shown in figure 52, *b*: Body 190 by

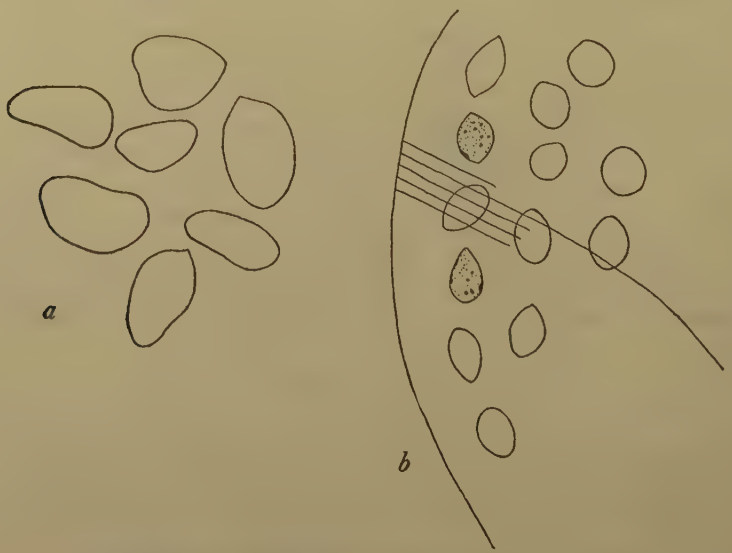

FIGURE 58.—*Cepedea buergeri sinensis* Metcalf: *a*, × 78; *b*, × 673.

120; nuclei 9.9 by 9.9, daughter nucleus 9.6 by 7, dividing nucleus 13.8 by 7.1; cilia line interval in front 2.2.

CEPEDEA BORNEONENSIS Metcalf

FIGURE 59

Host: *Bufo jerboa* Boulenger, from Trong, Lower Siam. One host (U.S.N.M. No. 24041), 28 mm. long, was abundantly infected.

The Siamese Cepedeas from this host are considerably flattened and are larger than the Bornean individuals. Their nuclei are for the

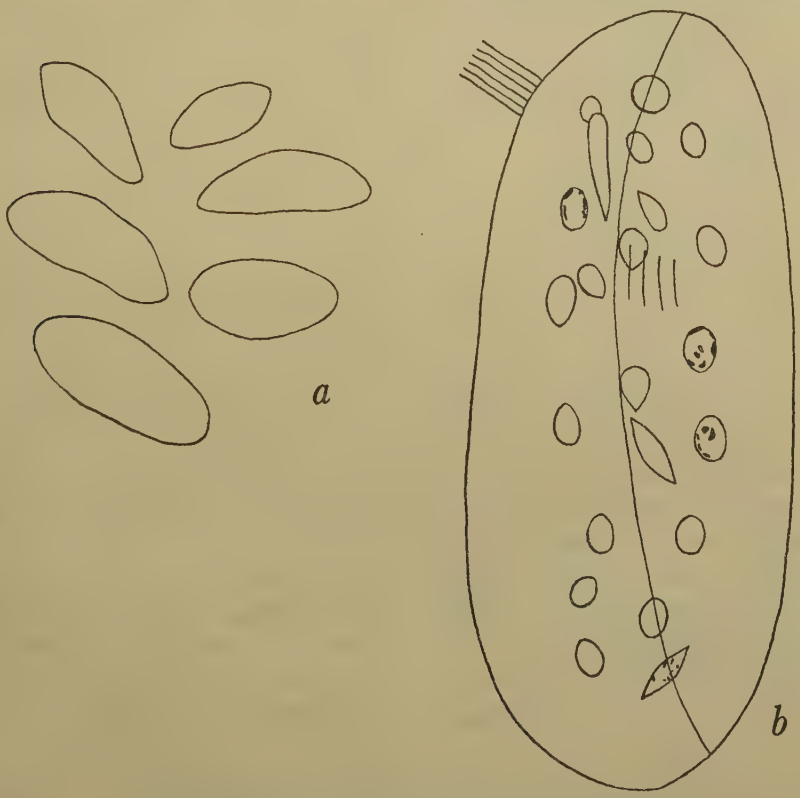

FIGURE 59.—*Cepedea borneonensis* Metcalf: *a*, × 117; *b*, × 1010.

most part elongated but less so than in the specimens from Borneo. In all the specimens from both localities the nuclei mostly lie with their long axis longitudinal. More or less flattening is not rare in the genus *Cepedea*, but this occurs in the elongated species more than in forms of this type.

Measurements, in microns: Body 100 by 38, 80 by 30, 60 by 29, 100 by 42; nuclei of last specimen 4.6 by 4.6, 6.3 by 2.9, 5 by 3.8, 9.9 by 3, 3.1 by 3.1; length of cilia in same specimen 9.8; cilia line interval 2.

CEPEDEA CELEBENSIS, new species

FIGURE 60

Type: U.S.N.M. No. 22632.

Host: *Bufo celebensis* Schlegel, from Teneboon, Celebes, East Indies, two specimens (U.S.N.M. Nos. 55395 and 55394), both infected, one very heavily.

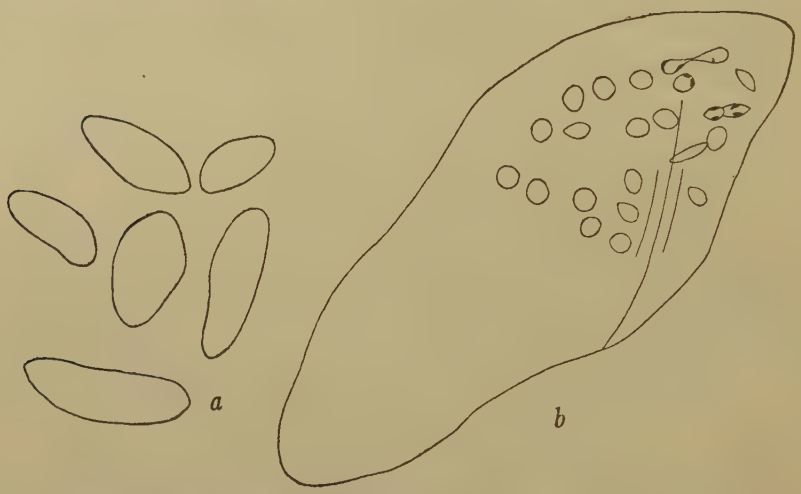

FIGURE 60.—*Cepedea celebensis*, new species, from *Bufo celebensis*: *a*, × 146; *b*, × 673.

Measurements, in microns: Body 134 by 40, 66 by 33, 120 by 50; nuclei spherical 4, 4.8, 3.9, dividing 9.9 by 2.1, 8.4 by 2.4, dumbbell-shaped 13 by 2.8; cilia line interval 2.5.

This species differs in shape and in range of shapes from *C. formosae* and *C. fujiensis*, and from *C. siamensis* in smaller size and relatively smaller nuclei and especially in the slender, much-elongated form of the dividing nuclei.

CEPEDEA CELEBENSIS

FIGURE 61

Host: *Bufo divergens* Peters, from Djambajan, Borneo, two specimens—U.S.N.M. No. 51727, 32 mm. long, uninfected, and No. 51725, abundantly infected.

Measurements, in microns: Body 131 by 45.3, 90 by 30, 107 by 36.6; nuclei 5 by 4.2, 4.7 by 3.2, half of dumbbell-shaped nucleus 7.4 by 2.8; length of cilia 8; interval between lines of cilia anteriorly 2.6; interval between cilia in a line 1.7.

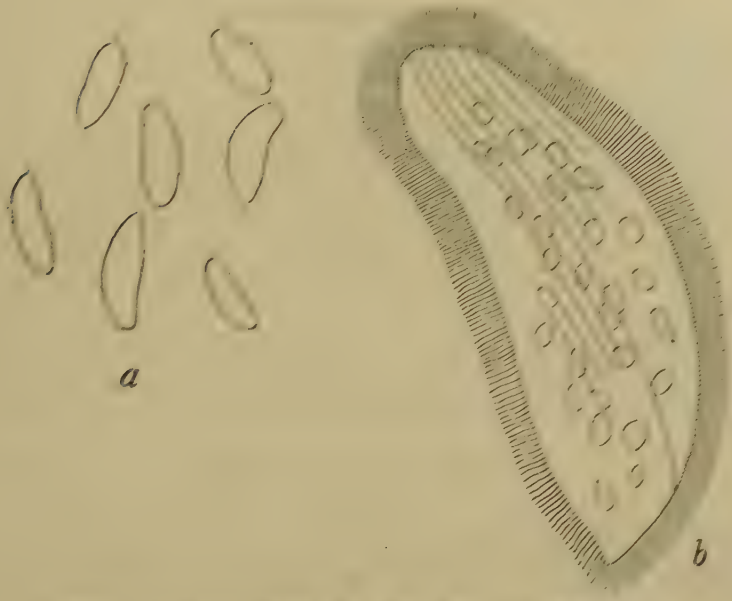

FIGURE 61.—*Cepedea vitiensis*. From *Rufo thoracus*: *a*, × 117; *b*, × 672.

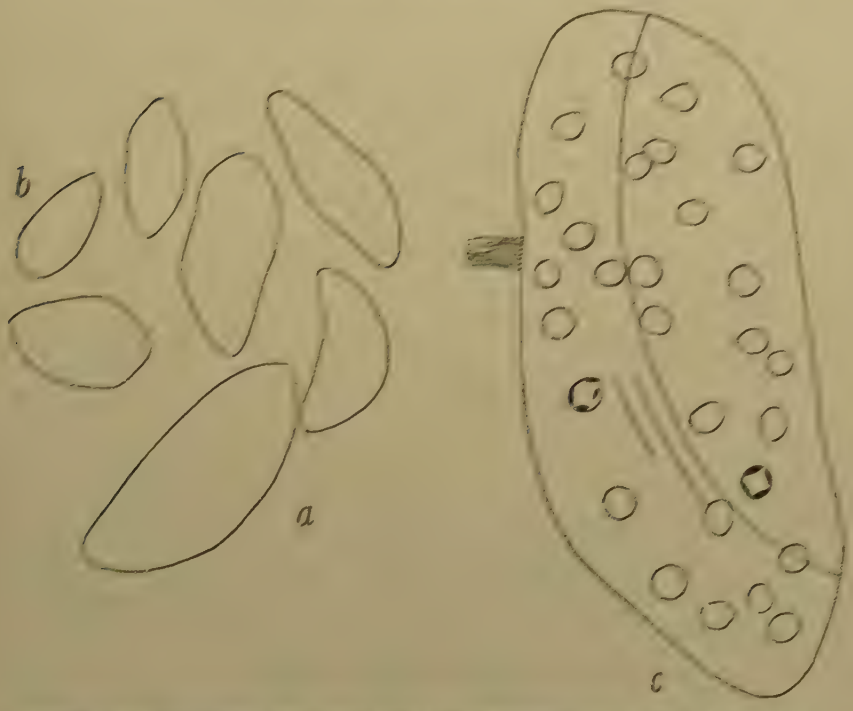

FIGURE 62.—*Cepedea bensleii*, new species: *a* and *b* and smear stained with thionin, × 266; *c*, × 1000.

CEPEDEA HASSELTII, new species

FIGURE 62

Type: U.S.N.M. No. 22633.

Host: *Leptobranchium hasseltii* Tschudi (=*Megophrys*).

A specimen of this pelobatid from Tamandjaija, Bantam, Java, collected by Bryant on January 19, 1909 (U.S.N.M. No. 62366), 80 mm. long, was fairly well infected with a rather small, stocky *Cepedea* with small, spherical nuclei. Another host of the same species (U.S.N.M. No. 3097), from eastern Borneo, 53 mm. long, bore no opalinids except numerous cysts.

Measurements, in microns: Body (fig. 62, *a*) 100 by 62, (fig. 62, *b*) 63 by 30, (fig. 62, *c*) 92 by 36.9; other measurements from figure 62, *c:* Dividing nucleus 3.5 by 3.5, spherical 4, 3.2; cilia line interval 2.5.

CEPEDEA MICROHYLAE, new species

FIGURE 63

Type: U.S.N.M. No. 22634.

Host: *Microhyla leucostigma* Boulenger.

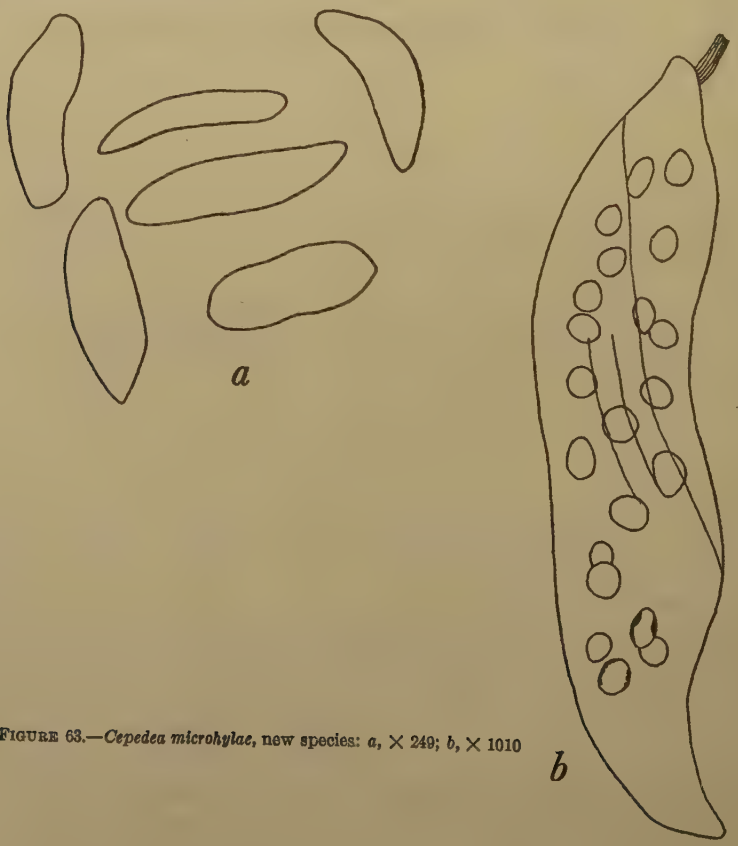

FIGURE 63.—*Cepedea microhylae*, new species: *a*, × 249; *b*, × 1010

A specimen of this gastrophrynid from Kuching, Sarawak, Borneo (U.S.N.M. No. 53650), 21 mm. long, bore numerous Cepedeas with rather small, spherical, or somewhat elongated nuclei and cilia of medium length in widely separated lines. They seem distinct.

Measurements, in microns: Body 130 by 36, 100 by 17, 100 by 20. Further measurements from last specimen: Nuclei 4, 5 by 3, 4 by 3.3, 5.8 by 2.3; length of cilia 6; interval between lines of cilia in anterior part of body 3.8.

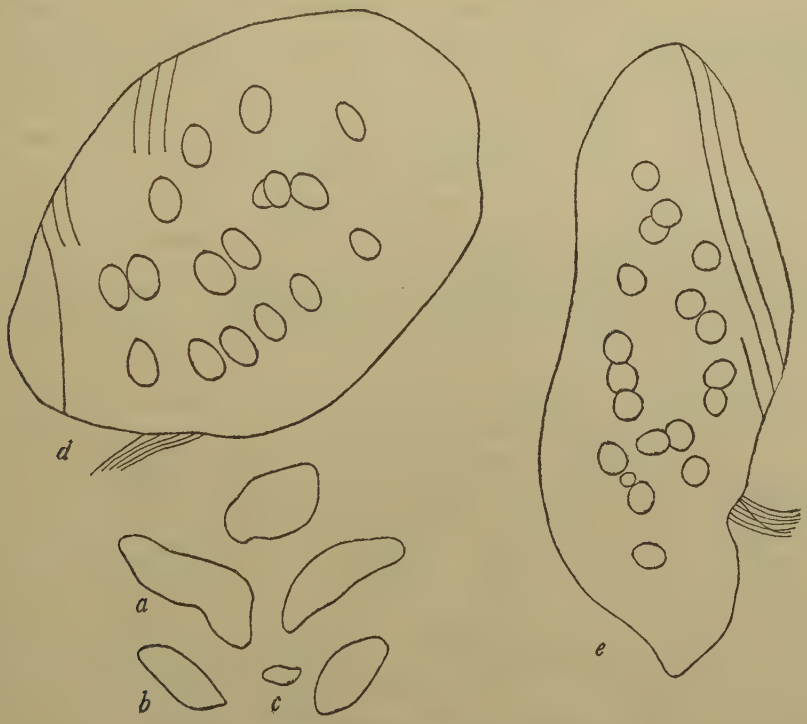

FIGURE 64.—*Cepedea hosei*, new species: *a–c*, × 146; *d* and *e*, × 678.

CEPEDEA HOSEI, new species

FIGURE 64

Type: U.S.N.M. No. 22635.

Host: *Nectophryne hosei* Boulenger. Two specimens from the Landak River, western Borneo, found in copulation, the male (U.S.N.M. No. 36315), 67 mm. long, well infected, the female (U.S.N.M. No. 36314), 100 mm. long, with no opalinids; two other specimens from the Kendawangan River, southeastern Borneo, 73 and 103 mm. long, the larger a female with eggs and bearing many opalinid cysts only, the smaller animal with no opalinids.

This *Cepedea* has long cilia in lines unusually far apart. Some individuals have nearly all nuclei elongated and large, probably approaching division; others show most of the nuclei smaller and about

spherical. In general appearance of an infection, in size and shape of individuals, and in the large nuclei they resemble the Asian-Malaysian group of species. About the only diagnostic distinction is the coarseness of the ciliation.

Measurements in microns:

Measurement	*a*	*b*	*c*	*d*	*e*
Body	116 by 34	80 by 29	100 by 70	100 by 70	100 by 43
Nucleus	----------	----------	----------	10 by 5.7	5.8 by 5.8
Nucleus	----------	----------	----------	9.8 by 6.4	5.1 by 5.1
Length of cilia	----------	----------	----------	14.5	13.8
Cilia line interval	----------	----------	----------	3	3.5

CEPEDEA SIAMENSIS, new species

FIGURE 65

Type: U.S.N.M. No. 22636.

Host: *Bufo asper* Gravenhorst, from Trong, Lower Siam, two specimens, one heavily infected. (U.S.N.M. No. 24033.)

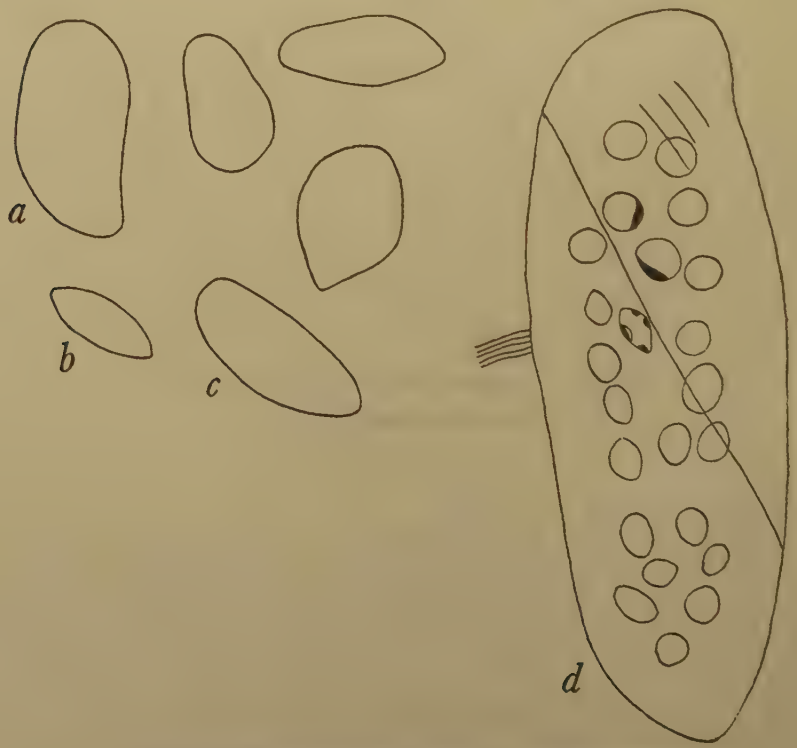

FIGURE 65.—*Cepedea siamensis*, new species: *a–c*, × 249; *d*, × 1010.

Measurements, in microns: Body (*a*) 116 by 58, (*b*) 64 by 24, (*c*) 100 by 40, (*d*) 94 by 32. Other measurements from *d*: Nuclei 5.2, 6 by 5, 6.5 by 4, 6.2 by 4.8, 4.8 by 3; length of cilia 7.1; interval between lines of cilia 2.6.

This *Cepedea* is somewhat like *C. fujiensis* but is only half as large, has nuclei a little smaller, and is more diverse in form. It also somewhat resembles *C. formosae* but is a third shorter, has nuclei of nearly twice the dimensions, and, again, is of more diverse shapes. It seems a distinct species but related to them both.

CEPEDEA VIRGULA (Dobell)

Opalina virgula Dobell, 1910.

Host: *Polypedates leucomystax* (Gravenhorst).

Dobell described as *Opalina virgula* opalinids from this host from Ceylon. He very kindly sent me a slide. I have infections in two specimens of this host from Tenasserim, Malay Peninsula (U. S. N. M. Nos. 34515 and 34516), each 41 mm. long, and one infection from Pulo Sianten, Anambas Islands (U. S. N. M. No. 26552), 70 mm. long, a tremendously heavy infection. Comparing these with Dobell's slide and description shows that they are the same and that they should be assigned to the genus *Cepedea*. This genus had not been created in the year 1910, when Dobell's paper appeared, and the wrong classification was my own error. These Cepedeas are mostly, but not always, unusually flat and might almost be regarded as Opalinas of the subgeneric group *Opalinae angustae*, but comparison with infections from other regions shows their affinities. A narrow *Opalina* from Ceylon was an anomaly, for the narrow species of this genus are North American or were derived from North America (e. g., *O. obtrigona* Metcalf, 1923a). The indications that this species is a *Cepedea* therefore solve a difficult puzzle.

CEPEDEA MOGYANA (Carini)

Figure 66

Opalina mogyana Carini, 1937.

Host: *Hyla leucophyllata* (Beiris), from Angra dos Reis, State of Rio de Janeiro, Brazil.

Of three specimens of this frog two were well infected with a *dimidiata*-like *Cepedea*. The lines of cilia are widely spaced, the nuclei of medium size. The nuclei contain spherical masses of nucleolar substance, which recall Weill's species *Opalina chattoni*, but no chromatin spireme, such as is in the latter species, was seen.

Measurements in microns: Body (*a*) 144 by 48, (*b*) 122 by 30. Other measurements from *a:* Nuclei 5.4, 4.5, 3.8; interval between lines of cilia in the anterior part of the body 3.1.

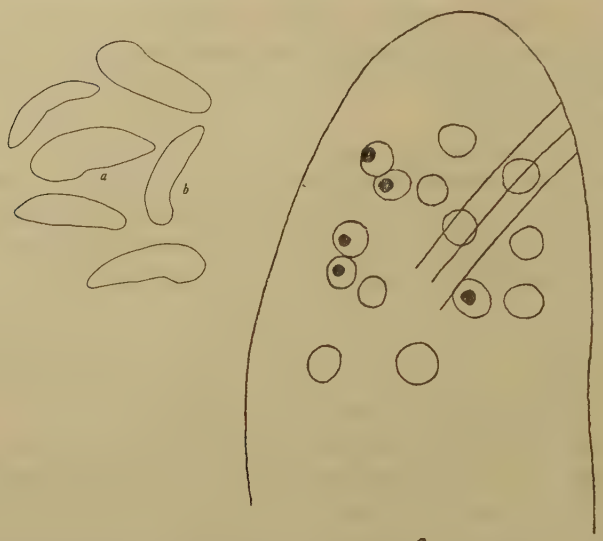

FIGURE 66.—*Cepedea mogyana* (Carini): *a* and *b*, × 117; *c*, × 1010.

CEPEDEA SPINIFERA Metcalf

Host: *Oxydozyga lima* (Tschudi).

This *Cepedea* in this host has been reported from Java (Metcalf, 1923a). The present abundant infections in the same species of host are from Baudon (U.S.N.M. No. 67244) and Lem Sing (U.S.N.M. No. 67313), Siam. Two other specimens from Siam were uninfected.

CEPEDEA LEMURIAE, new species

FIGURE 67

Type: U.S.N.M. No. 22637.

Host: *Polypedates rhodoscelis* (Boulenger).

A single specimen of this tree frog from Madagascar (U.S.N.M. No. 60658), 38 mm. long, a female with eggs, is abundantly infected with rather large Cepedeas, which, as in *C. dimidiata*, show small, slender individuals and also very much swollen larger individuals. These all have small nuclei. The individual marked *x* in the figure shows an irregularity of contour, indicating probably a thin pellicle and soft, flabby body. Such individuals are rather numerous in the infection, as they are in infections of *C. multiformis* (tropical America), *C. seychellensis* (Seychelles Islands), *C. hispanica* (Spain), *C. minor* (France), and some others. This species has a combination of characters not seen in any other *Cepedea* described and is named as a distinct species, after the former Indian Ocean continent of which Madagascar is reputed to have been a portion.

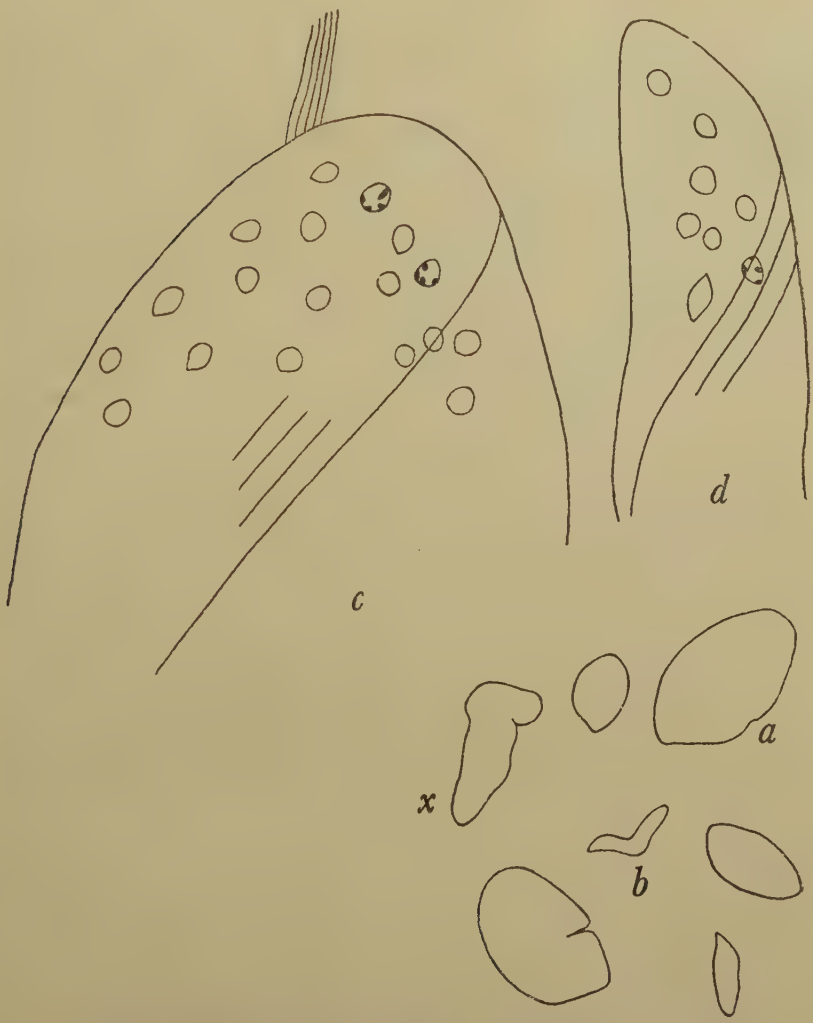

FIGURE 67.—*Cepedea lemuriae*, new species: *a*, *b*, and *x*, × 117; *c* and *d*, × 1010.

Measurements, in microns:

Measurement	*a*	*b*	*c*	*d*
Body	200 by 73	110 by 20		
Nucleus			3.3 by 3.3	6.5 by 2.8
			3.9 by 3.9	3.7 by 3.7
			3 by 3 (daughter nucleus)	3.5 by 2.3
			5 by 3 (dividing nucleus)	
Length of cilia			10	
Interval between lines of cilia			3.1	3

CEPEDEA RUBRA (Carini)

FIGURE 68

Opalina rubra CARINI, 1937.

Host: *Hyla minuta* Peters, from mountains near Rio de Janeiro, Brazil.

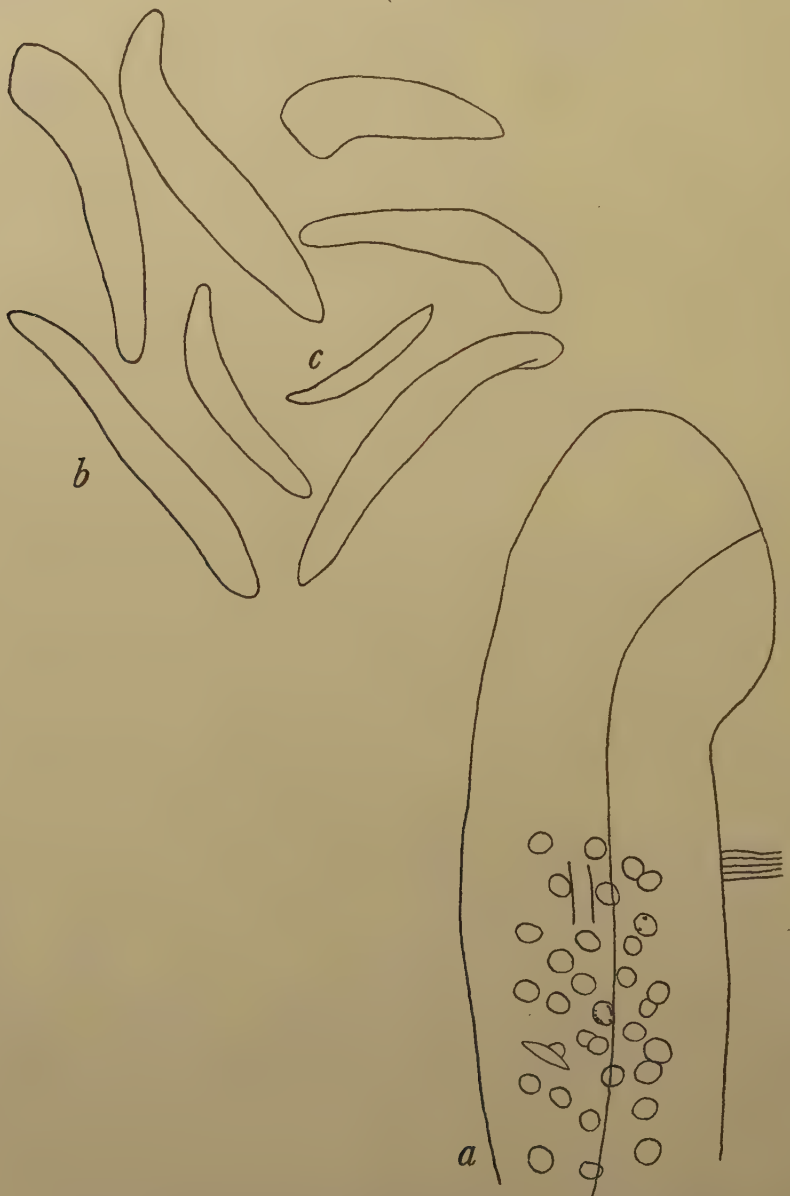

FIGURE 68.—*Cepedea rubra* (Carini) from *Hyla minuta*: *a*, × 1010; *b* and *c*, × 249.

Two out of five specimens, 22 mm. long, were infected with a rather flat *Cepedea*. Its nuclei are very small, their diameter about equal to the interspace between the lines of cilia. *C. rubra* is longer than *C. dimidiata* of any subspecies. In *C. paraguensis* (Metcalf's *C. dimidiata paraguensis*, 1923a) the smallest nuclei are about as large as the largest in *C. rubra*, but the larger are twice as large as the usual ones in the latter species. The appearance of the infections as a whole in the two species, the shapes, and the percentages of individuals of the several shapes, are different.

Measurements, in microns: Figure 68, *a*, width of body 34.6, nucleus 3.1, 2.1, dividing nucleus 7.6 by 1.7; length of cilia 7.7; cilia line interval 2.7. Figure 68, *b*, body 200 by 23. Figure 68, *c*, body 90 by 12.

CEPEDEA RUBRA (Carini)

FIGURE 69

Host: *Pseudopaludicola ameghini* (Cope), from Minas Geraes, Brazil, 7 specimens, 4 uninfected, 3 showing heavy infections.

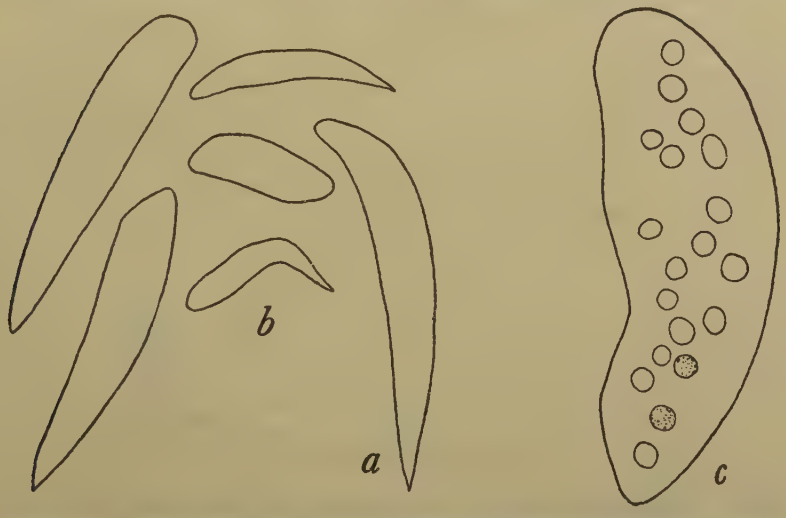

FIGURE 69.—*Cepedea rubra* (Carini) from *Pseudopaludicola ameghini: a* and *b*, × 249; *c*, × 1010.

These frogs were all dead, perhaps 18 hours or so, before they were opened. Probably in consequence of this, the Cepedeas were very slow in their movements and were much twisted spirally. These belong to the group of more or less elongated species with soft pellicle and soft bodies. They so intergrade as to be difficult to distinguish. The present specimens seem the same as the forms in *Hyla minuta*.

Measurements in microns: (*a*) Body 200 by 30; (*b*) body 90 by 17; (*c*) body 64 by 22; nuclei 3.4, 3, 2.7; dividing nuclei 4.9 by 3.

CEPEDEA RUBRA (Carini) (?)

FIGURE 70

Host: Tadpole of *Leptodactylus ocellatus* (Linnaeus), from Manguinhos, Rio de Janeiro, Brazil.

Three stages of development of this opalinid are shown in the drawings: The youngest, *a*, in a *Protoopalina axonucleata* condition; *b*, a somewhat older larva whose nuclei are beginning to be irregularly distributed through the cytoplasm; and *c*, approaching the adult

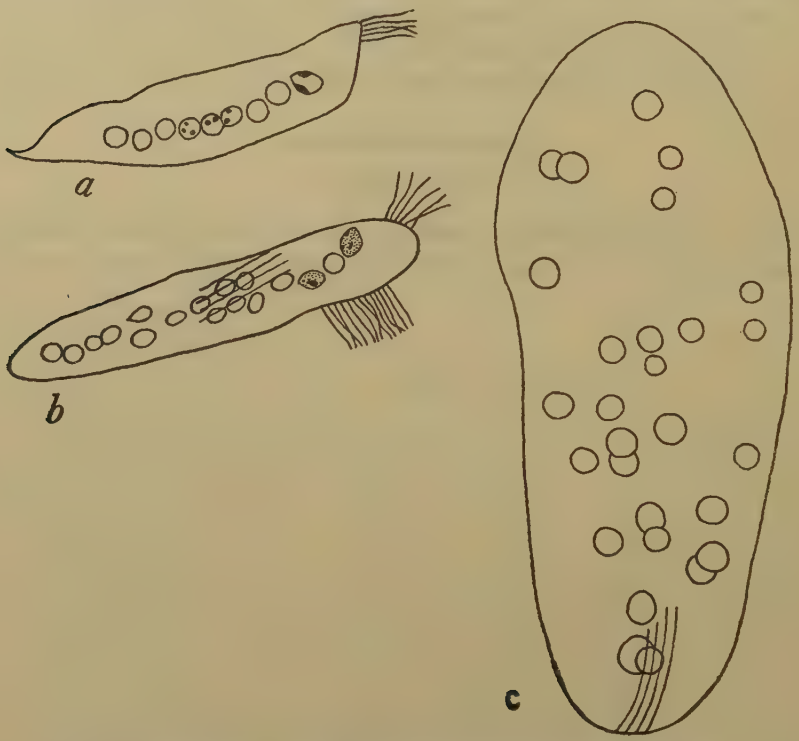

FIGURE 70.—*Cepedea rubra* (Carini) (?) from tadpole of *Leptodactylus ocellatus*, × 820.

condition. The small size of the nuclei suggests resemblance to *C. rubra* rather than to *C. longa*, and this is emphasized when we remember that in the development of *Cepedea* the young individuals have proportionally larger nuclei than the old. *C. dimidiata* [*paraguensis*] Metcalf has still smaller nuclei, much smaller. But the assignment of these larvae to the species *rubra* without seeing an adult is of most doubtful validity, but it seems the most probable of the three species known to occur in the region.

Measurements in microns:

Measurement	*a*	*b*	*c*
Body length	60	68	114
Body width	14	11	45
Diameter of nucleus	3. 6	2.4 to 3	3.6 to 5, average 4.6
Length of cilia	9. 7		
Interval between lines of cilia		1. 5	1. 5

CEPEDEA PHILIPPENSIS, new species

FIGURE 71

Type: U.S.N.M. No. 22638.

Host: *Bufo philippinicus* Boulenger, from Caiholo River, Ulugan Bay, Palawan, Philippine Islands (U.S.N.M. No. 39965).

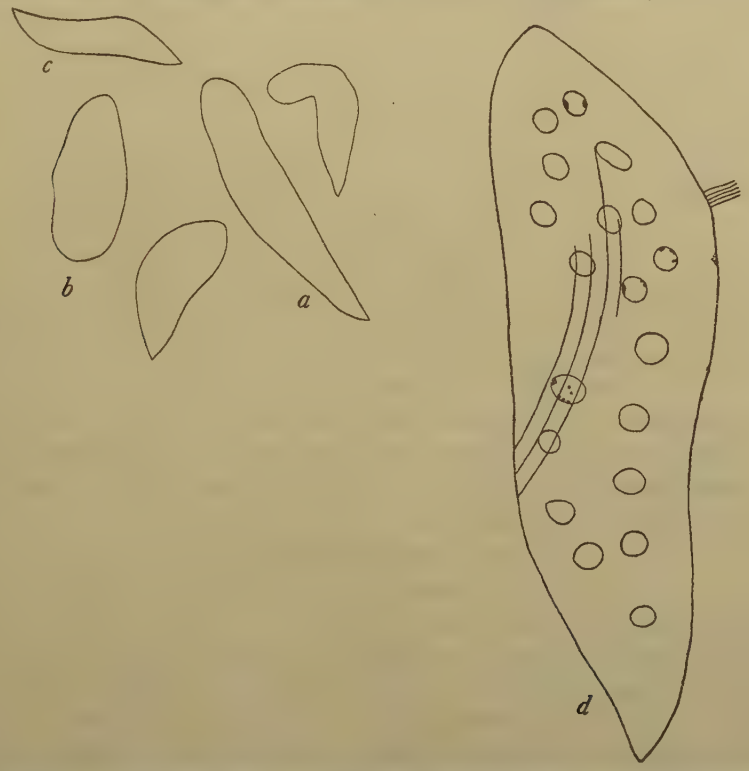

FIGURE 71.—*Cepedea philippensis*, new species, from *Bufo philippinicus*: *a–c*, × 146; *d*, × 673.

Measurements, in microns: Body (*a*) 230 by 48, (*b*) 138 by 57, (*c*) 140 by 30, (*d*) 140 by 40. Other measurements from *d:* Spherical nuclei, 4.7 to 5.7 in diameter, dividing 8.4 by 4; length of cilia 7.4; cilia line interval 2.3.

These Cepedeas seem to belong to the Asian-Malaysian group containing *C. formosae*, *C. fujiensis*, *C. siamensis*, and *C. celebensis*.

They most resemble *C. formosae* in shape and size and in the dimensions of the nuclei, but the dividing nuclei are much more elongated, as they are in *C. celebensis*. It seems to be another case of intergrading species.

CEPEDEA PHILIPPENSIS

FIGURE 72

Host: *Bufo quadriporcatus* Boulenger, from Pulo Sugi, Rhio Archipelago, south of western Sumatra, 2 specimens (U. S. N. M. Nos. 30986 and 30987), both infected.

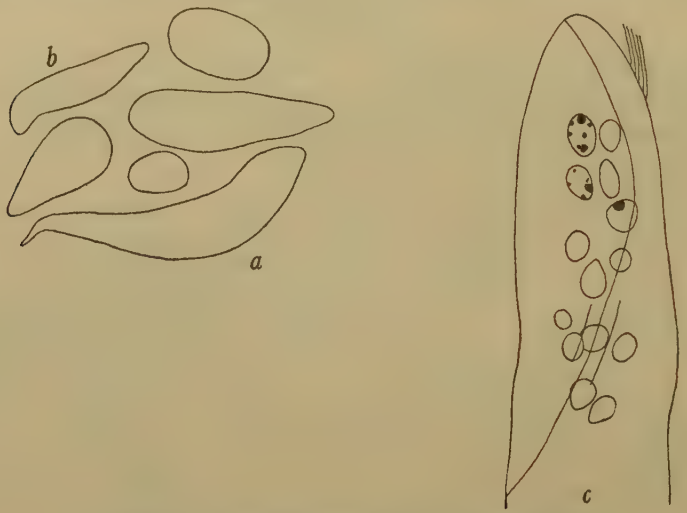

FIGURE 72.—*Cepedea philippensis: a* and *b*, × 146; *c*, × 673.

Measurements, in microns: Body (*a*) 229 by 50, (*b*) 128 by 29; (*c*) width of body 30. Other measurements from *c:* Nucleus 5.4 by 4.3, daughter nucleus 7.3 by 4.3, dividing nucleus 8 by 5; length of cilia 9.9; cilia line interval 2.5.

CEPEDEA LUZONENSIS, new species

FIGURE 73

Type: U.S.N.M. No. 22639.

Host: *Rana luzonensis* Boulenger, one specimen (U.S.N.M. No. 38047), 60 mm. long, from Benquet Province, Philippine Islands, collected July 1, 1907, and annotated "Heights in the Oaks."

This frog bore a generally slender *Cepedea* whose forms of body, forms and sizes of nuclei and ciliation do not agree with any other species described. The general impression from the appearance of an infection is distinct, and this is one of the most reliable indications even when indefinable.

Measurements, in microns: Body (*a*) 240 by 60, (*b*) 180 by 55, (*c*) 60 by 31, (*d*) width of body 36. Other measurements from *d:* Nuclei 4, 4.9 by 4, dividing nuclei 5.9 by 4, length of cilia 8.9, interval between lines of cilia 2.7. The number of nucleoli is probably 4.

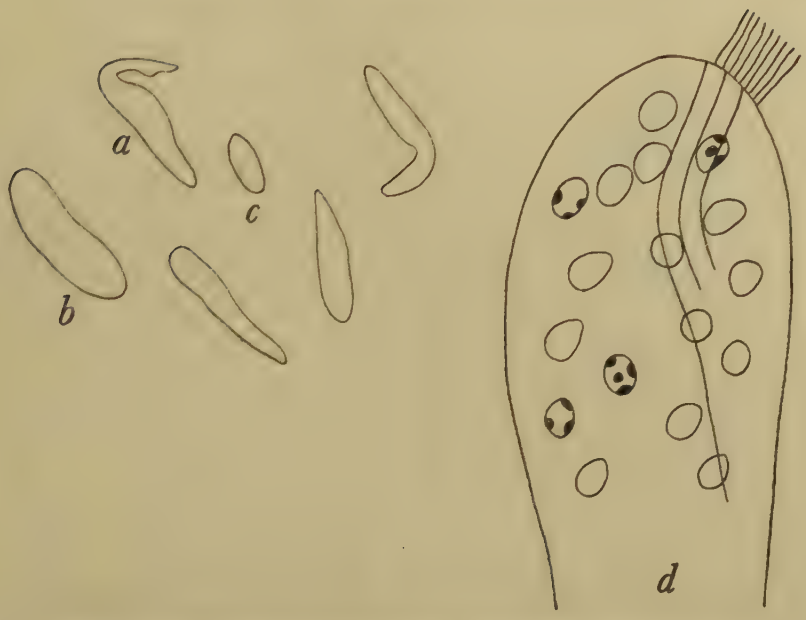

FIGURE 73.—*Cepedea luzonensis*, new species, from *Rana luzonensis*: *a-c*, ×117; *d*, ×1010.

CEPEDEA LUZONENSIS

FIGURE 74

Host: *Rana similis* Günther, one specimen, from Rizal, Philippine Islands, well infected.

These Cepedeas are evidently the same as those in *Rana luzonensis*.

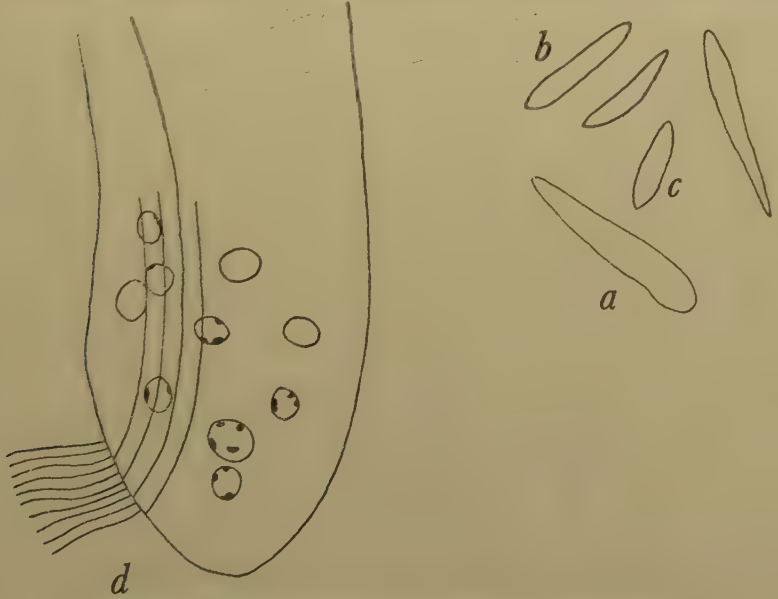

FIGURE 74.—*Cepedea luzonensis*, from *Rana similis*: *a-c*, ×117; *d*, ×1010.

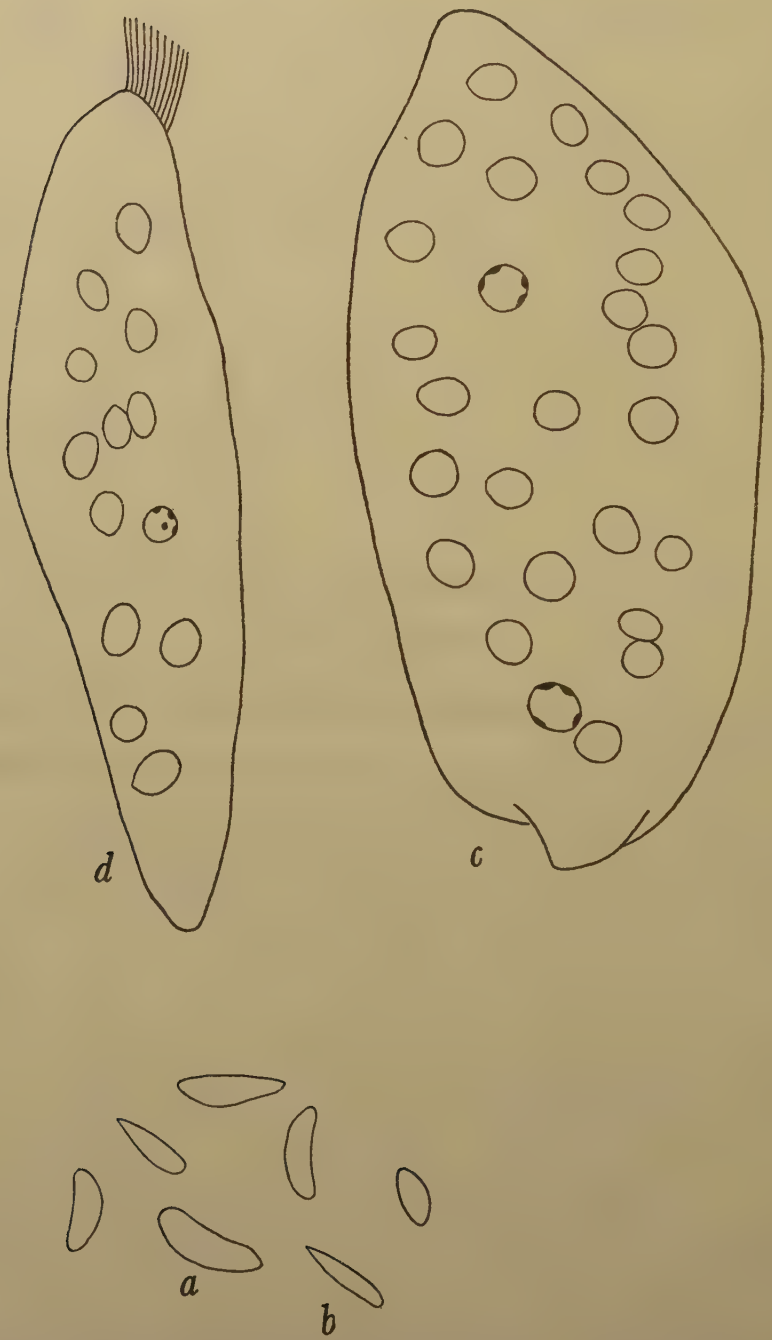

FIGURE 75.—*Cepedea luzonensis aponensis*, new subspecies, from *Rana magna*: *a* and *b*, ×117; *c* and *d*, ×1010.

CEPEDEA LUZONENSIS APONENSIS, new subspecies

FIGURE 75

Type: U.S.N.M. No. 22640.

Host: *Rana magna* Stejneger, two specimens, from Mount Apo, Mindanao, Philippine Islands (U.S.N.M. Nos. 34778 and 34780).

Both specimens of the host were well infected with a *Cepedea* smaller than *C. luzonensis*. The shapes of the individuals and the proportions of the different shapes in the infections remind one strongly of some infections of *C. dimidiata* in which the swollen

FIGURE 76.—*Cepedea ciliata*, new species, from *Hyla fuscovaria*: *a* and *b*, ×117; *c*, ×1010.

forma *zelleri* is not present. It apparently belongs with *C. dimidiata* in the same subgenus, as does also *C. luzonensis*. As good a treatment as I can suggest is to class it as a subspecies of *C. luzonensis*.

CEPEDEA CILIATA, new species

FIGURE 76

Type: U.S.N.M. No. 22641.

Host: *Hyla fuscovaria* Lutz, from the State of Minas Geraes, Brazil; two specimens, one uninfected.

The infected specimen of this frog bore myriads of Cepedeas intermediate in shape between the *longa* type and the *dimidiata* type. They are considerably flattened. The most marked character is a very dense ciliation, the spaces between the rows of cilia in the anterior portion of the body being only 1μ.

Body measurements, in microns: 30 by 44, 154 by 34, 140 by 36.

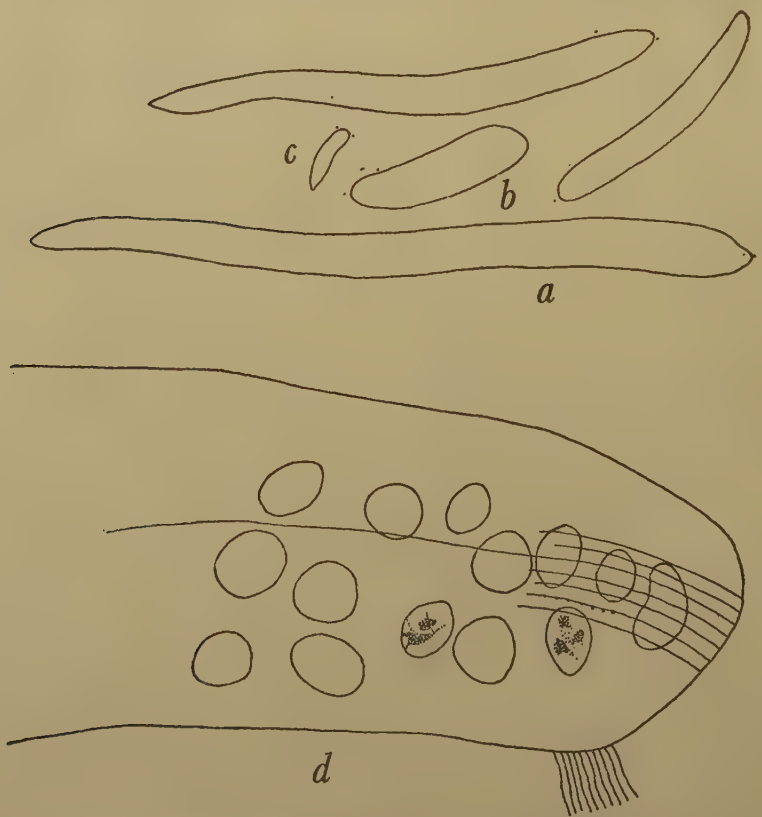

FIGURE 77.—*Cepedea longa macronucleata*, new subspecies, from *Rana vittigera*: *a*–*c*, ×117; *d*, ×1010.

CEPEDEA LONGA MACRONUCLEATA, new subspecies

FIGURE 77, 78

Type: U.S.N.M. No. 22642.

Host: *Rana vittigera* Wiegmann,[4] from Manila, Philippine Islands, four specimens, three uninfected, the fourth abundantly infected (U.S.N.M. No. 39175), 44 mm. long; also a specimen from Guijulugan, Negros Island (U.S.N.M. No. 68655), 44 mm. long, very heavily infected.

Measurements, in microns: Body (*a*) 800 by 60, (*b*) 166 by 55, (*c*) 70 by 18.8; other measurements (*d*): Width of body 46, nuclei 7.6,

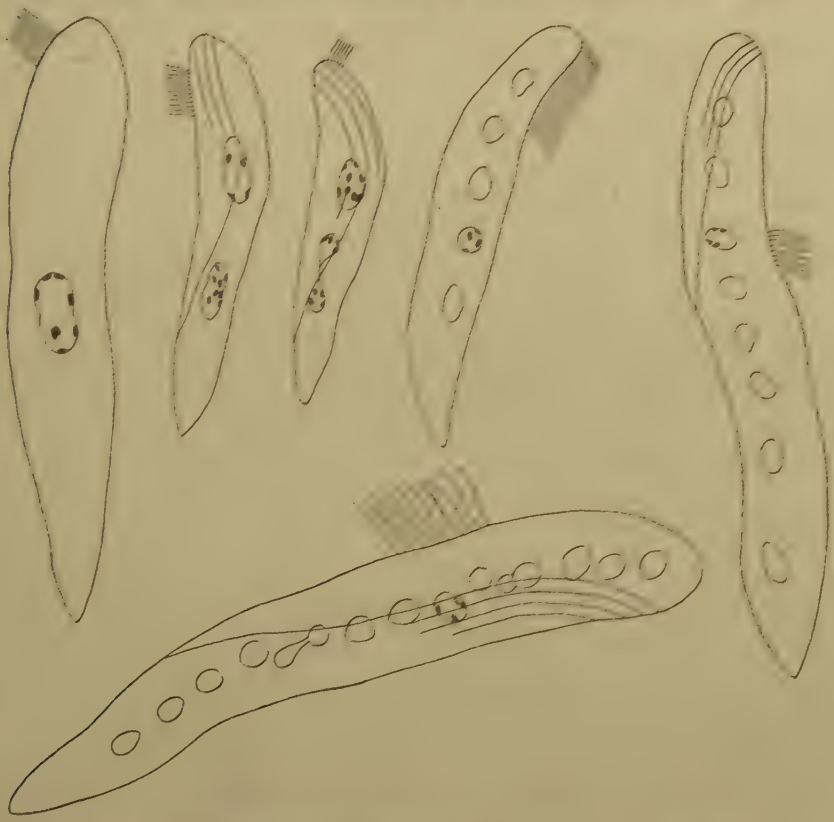

FIGURE 78.—Larvae of *Cepedea longa macronucleata*, from tadpoles of *Rana vittigera*, ×673.

9.7 by 7, 8.7 by 5.5, 6.9 by 5, cilia length 8.4, interval between cilia lines 1.7; nucleoli 4.

In two tadpoles (U.S.N.M. Nos. 39252A and 39252B), 11.5 and 12 mm. long, respectively, were found abundant larval Cepedeas (fig. 78) showing a series of stages in development. The uninucleate and binucleate ones are essentially Protoopalinas. As the nuclei increase in number they remain in a line down the axis of the body,

[4] Perhaps equivalent to *R. cancrivora* of the Malay Archipelago

recalling *P. axonucleata* (Metcalf, 1923a). Later the nuclear arrangement becomes irregular. In the dividing nuclei the nucleolus number is clearly seen to be 4. In most of these larval nuclei, though not in division, the nucleolar masses are 4. No flattened larvae are seen, that is, there is no *Zelleriella* stage in the development of *Cepedea*. All this agrees with the phylogeny as I postulated it on the basis of comparative anatomy, deriving *Cepedea* from *Protoopalina* through forms like *P. axonucleata*.

CEPEDEA LONGA HISPANICA Metcalf

FIGURE 79

Cepedea hispanica METCALF, 1923a, p. 161.

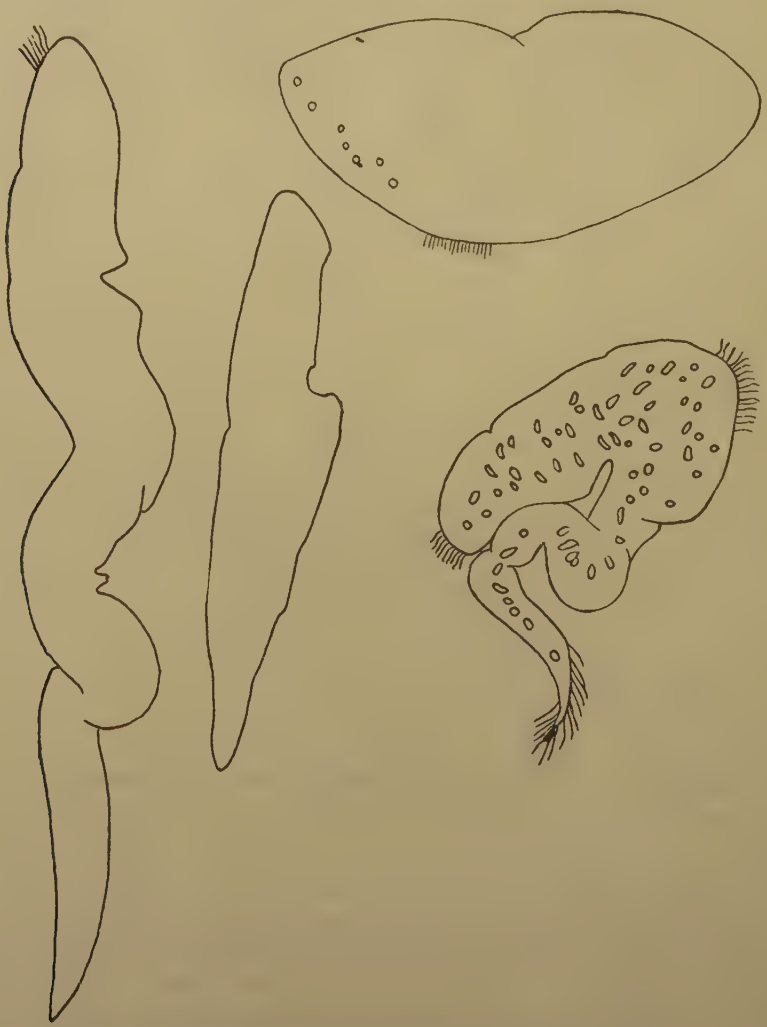

FIGURE 79.—*Cepedea longa hispanica* Metcalf from *Rana limnocharis*, × 460.

Host: *Rana limnocharis* Wiegmann, one specimen, from Sitong Ridge, Darjiling District, northern India, abundantly infected.

Both the Indian and the Spanish parasites are very similar to *C. longa* but are not nearly so long. The nuclei are elongated with their long dimension across the body, as in *C. longa*. Some of the Darjiling individuals show irregular shapes owing to repeated divisions, indicating approach to the time of formation of gametes. In the infection are a few much swollen individuals. It seems best to demote *C. hispanica* from specific rank (Metcalf, 1923a), to class it as a subspecies of *C. longa*, and to include the Darjiling specimens in spite of their having some peculiar, swollen individuals. Many species of *Cepedea* have occasional enlarged forms.

Measurements, in microns: Body (*a*) 166 by 21.7, (*b*) 315 by 26.1, (*d*) 370 by 26. Other measurements of *c:* Nucleus 5 by 1.6, 4 by 1.2, daughter nucleus 3, cross diameter of dividing nucleus 1.5; length of cilia 6.

CEPEDEA PLATA, new species

FIGURE 80

Type: U.S.N.M. No. 22643.

Host: *Hyla faber* Wied.

One of four specimens of this tree frog from Rio de Janeiro, Brazil, and each of two from Angra dos Reis, in the same State, bore enormous infections of this remarkably flat *Cepedea*.

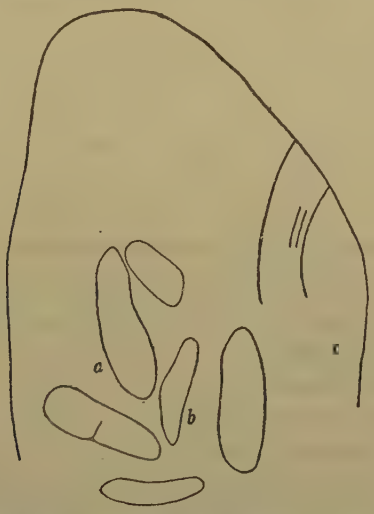

FIGURE 80.—*Cepedea plata*, new species, from *Hyla faber: a* and *b*, × 117; *c*, × 505.

Measurements, in microns: Length of body (*a*) 350, (*b*) 240: interval between lines of cilia 1.7.

CEPEDEA (?)

FIGURE 81

Host: *Rana crassa* Jerdon.

In a rather young tadpole of this frog, 43 mm. long, body 13.5 mm. and hind legs 2 mm. long, collected in southern India, were opalinid larvae that, of course, could not be identified without a larger series

FIGURE 81.—*Cepedea* (?) from tadpole of *Rana crassa*, × 820.

of material. They seem to be *Cepedea*, though there is a possibility that they are *Opalina* in a *Cepedea* stage of the life history.

Measurements, in microns: Body length 77, width 16; nuclei 3.4 by 3.2, 4.7 by 2.6, 3.8 by 3.8.

CEPEDEA species (?)

FIGURE 82

Host: *Eleutherodactylus guentheri* (Steindachner), from Angra dos Reis, State of Rio de Janeiro, Brazil.

In tadpoles of this host, 46 mm. long, of which 17 mm. is length of body and the rest tail, there are very slender larvae of *Cepedea*, which

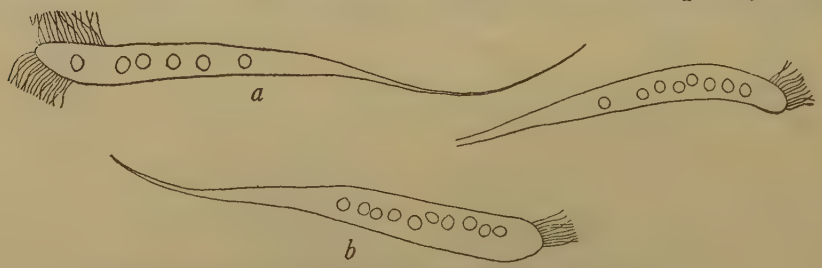

FIGURE 82.—*Cepedea* sp. (?) from tadpole of *Eleutherodactylus guentheri*, × 453.

cannot, from the material in hand, be assigned to any species. Note that, as is often the case in multinucleate opalinids, the nuclei are larger in the younger forms.

Measurements, in microns:

Measurement	*a*	*b*
Total length	160	130
Length of body	89	73
Length of tail	71	57
Length of cilia	11. 7	10
Width of body	10	13
Diameter of nucleus	5	3. 7

CEPEDEA species (?)

FIGURE 83

Host: Tadpoles of *Eleutherodactylus* sp. (?).

In a metamorphosing tadpole of an undetermined species of this frog, 56 mm. long and with hind legs 21 mm. and forelegs 11.5 mm. long, occurred Cepedeas as shown in figure 83.

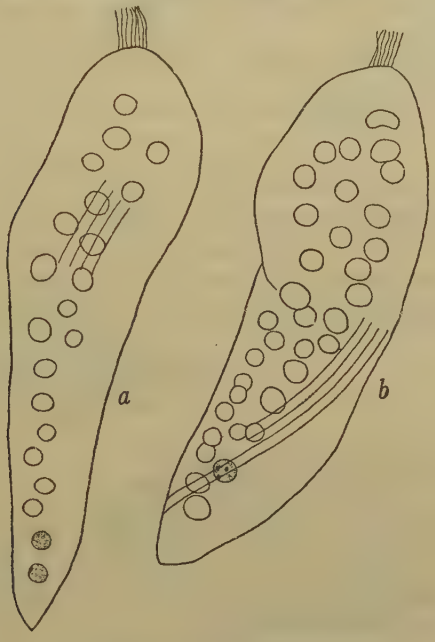

FIGURE 83.—*Cepedea* sp. (?) from tadpoles of *Eleutherodactylus* sp. (?), × 453.

Measurements, in microns:

Measurement	*a*	*b*
Length of body	183	120
Greatest width of body	44	50
Diameter of nuclei, average	2.4	2.4
Length of cilia	12	----
Interval between lines of cilia	3.3	2.7
Interval between cilia in line	1.4	----

CEPEDEA SCALPRIFORMIS Ghosh

FIGURE 84

Host: *Bufo melanostictus* Schneider, from India.

Resembles *C. dimidiata* in outline but quadrangular, wedge-shaped, and truncate in front. This seems a valid species.

FIGURE 84.—*Cepedea scalpriformis* Ghosh.

CEPEDEA SIALKOTI Bhatia and Gulati

FIGURE 85

Host: *Bufo macrotis* Boulenger, from Punjab, India.

In their drawings of this and other opalinids, Bhatia and Gulati (1927) have shown the rows of cilia as converging to the two ends of the body. Others have not observed this arrangement in any species. Probably it is due to inadvertent error of observation.

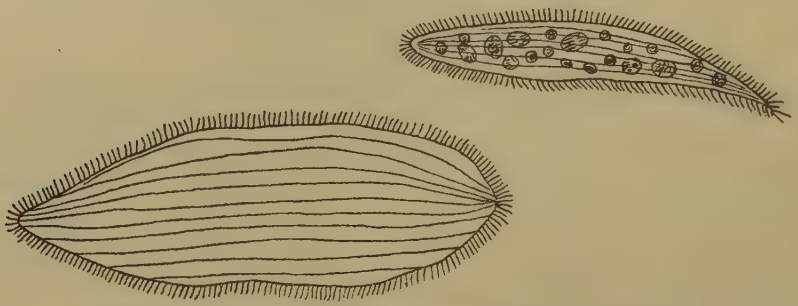

FIGURE 85.—*Cepedea sialkoti* Bhatia and Gulati.

Measurements, in microns, are recorded as follows: Body 89 by 31, 64 by 14, nucleus 7.

As in many other species, broad and narrow forms are found (cf. *C. dimidiata* and the forma *zelleri*). Without seeing specimens of this *Cepedea* I hesitate to discuss its affinities.

CEPEDEA METCALFI Bhatia and Gulati

FIGURE 86

Host: *Bufo melanostictus* Schneider, from India.

In this species, again, slender and stocky forms are found.

Measurements, in microns, are given as follows: Body 108 by 40, 85 by 35, 81 by 67, 71 by 17; interval between lines of cilia 2.5, apparently in the middle of the body. Nuclei spherical.

The description is not sufficient to distinguish this form from *C. sialkoti* or to allow determination of relationships.

FIGURE 86.—*Cepedea metcalfi* Bhatia and Gulati.

CEPEDEA PUNJABENSIS Bhatia and Gulati

FIGURE 87

Host: *Bufo melanostictus* Schneider, from Punjab, India.

The triangular shape of the anterior part of the body and its anterior point indicate this as a distinct species.

Measurements, in microns, are given as follows: Body 82 by 53; nucleus 4.

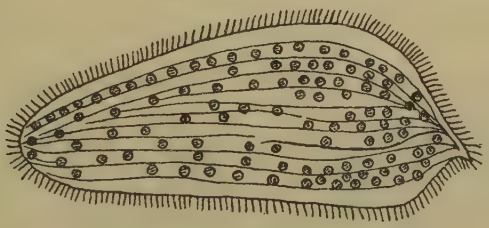

FIGURE 87.—*Cepedea punjabensis* Bhatia and Gulati.

The drawing shows the small nuclei in rows between the lines of cilia, an arrangement not before observed and difficult to understand, for the nuclei lie at a much deeper level. Probably the observation was not quite accurate.

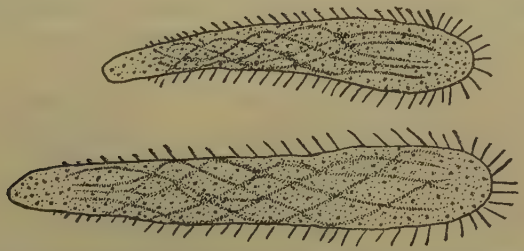

FIGURE 88.—*Cepedea rugosa* (Carini) from *Hyla nebulosa*, × ca. 220. (After Carini.)

CEPEDEA RUGOSA (Carini)

FIGURE 88

Opalina rugosa Carini, 1937.

Host: *Hyla nebulosa* Spix, from Brazil.

The body is generally straight or slightly bent, very slightly flattened, and measures 270–320μ in length by 30–45μ in width. The anterior end is a little larger than the posterior, which is blunt. This *Cepedea* has been found only once or twice in spite of numerous examinations. It presents on its surface a number of wrinkles, which begin near the anterior end and extend almost to the posterior end. In the anterior part the wrinkles have a linear longitudinal arrangement, but as they proceed toward the posterior end they intertwine. This wrinkled aspect is very characteristic of the species, seen in living condition immediately after they are removed from the intestine. It is not certain as yet whether this characteristic is a normal one. (A restudy of this species is desirable.)

CEPEDEA THIAGI de Mello

FIGURE 89

Host: *Rhacophorus maculatus* Gray, from Nova Goa.

One very distinct characteristic of this species of *Cepedea* is the alveolar appearance of the anterior end. The posterior end may be

FIGURE 89.—*Cepedea thiagi* de Mello from *Rhacophorus maculatus*. Magnification not given. (After de Mello.)

either rounded or pointed. Length of body varies from 125μ to 440μ. There are numerous nuclei having a diameter of 4–5μ.

FIGURE 90.—*Cepedea subcylindrica* de Mello from *Bufo melanostictus*. Magnification not given. (After de Mello.)

CEPEDEA SUBCYLINDRICA de Mello

FIGURE 90

Host: *Bufo melanostictus* Schneider, from Nova Goa.

This multinucleate *Cepedea* is elongated and spindle-shaped; the anterior end is generally less pointed than the posterior end. Variations are found in some animals; both the anterior and posterior ends are blunt—they appear as cylinders. Several drawings are copied from de Mello's paper, and they are diagrammatic although made with the aid of a camera lucida.

The measurements of a number of individuals show that they vary in length from 35μ to 250μ and in width from 15μ to 80μ; the diameter of the nuclei is 2.5–3.5μ. (The description is too scant for specific identification.)

CEPEDEA species (?)

Cepedeas of an unidentified species were found in a Siamese specimen of *Rana cancrivora* Gravenhorst (U. S. N. M. No. 66550). It was a scant infection and no drawings were made. Another specimen (U. S. N. M. No. 66551) was uninfected.

CEPEDEA species (?)

FIGURE 91

Host: *Aelurophryne mammata* Günther, from Songpau, Szechwan, western China.

Specimens of this toad, 21 and 22 mm. long, were lightly infected with Cepedeas too poorly preserved for study. In one host the parasites averaged 66μ long; in the other they were mostly about 190μ in length by 36μ wide. In form both the large and the smaller Cepedeas resemble *C. dimidiata* (Stein). Staining proved peculiarly difficult, so that no further report, upon nuclei or any other features, can be made.

FIGURE 91.—*Cepedea* sp. (?) from *Aelurophryne mammata*, × 250.

Genus OPALINA Purkinje and Valentin

OPALINA RANARUM ORBICULATA, new subspecies

FIGURE 92

Type: U.S.N.M. No. 22644.

Host: *Rana glandulosa* Boulenger.

One specimen of this host from Singapore (U.S.N.M. No. 34514) was very heavily infected with large Opalinas of the general *ranarum* type, though its nuclei run somewhat smaller. In the great complex

of *ranarum*-like forms it is difficult, probably impossible, to give any classification that will express relationship. Forms of similar appearance are not necessarily genetically close together.

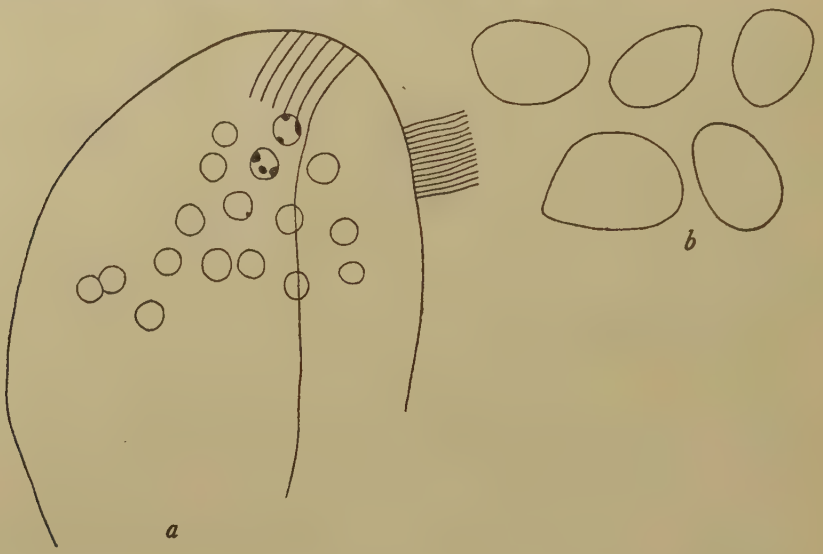

FIGURE 92.—*Opalina ranarum orbiculata*, new subspecies, from *Rana glandulosa*: *a*, × 673; *b*, × 78.

Measurements, in microns: Body 230 by 160, 180 by 100; width of body (in another specimen) 80; nuclei 6.4, 5.4, 4.9, 4.3; length of cilia 12.9; interval between lines of cilia 2.3.

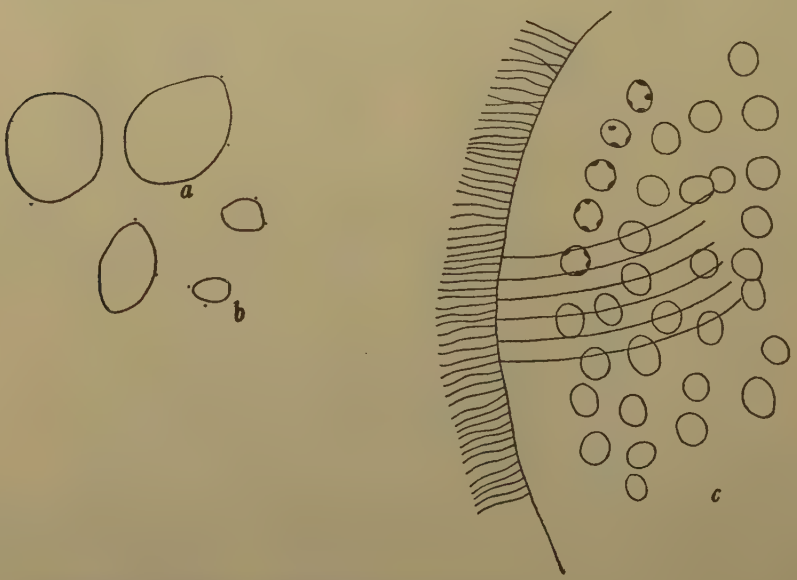

FIGURE 93.—*Opalina ranarum orbiculata* from *Rana temporalis*: *a* and *b*, × 78; *c*, ×673.

OPALINA RANARUM ORBICULATA

FIGURE 93

Host: *Rana temporalis* Günther, from Bogawantalava, Ceylon, 4,000 feet altitude.

Of six individuals opened, two (U.S.N.M. Nos. 67057 and 67058) were well infected. The Opalinas were large and broad, almost orbicular, their nuclei spheroidal, of moderate size, their cilia sparse, the lines being widely spaced even at the anterior end. They resemble the *ranarum* group rather than *japonica* in the shape of their posterior ends.

Measurements, in microns: (*a*) Body 210 by 160; (*b*) body 60 by 40, (*c*) nuclei 6.9, 6.5, 5.8, 4.7, length of cilia 10, interval between lines of cilia 4. Nucleoli 4.

OPALINA ZEYLONICA, new species

FIGURE 94

Type: U.S.N.M. No. 22645.

Host: *Polypedates eques* (Günther).

Two out of five specimens (U.S.N.M. No. 6790, 37 mm. long, and No. 67902, 19 mm. long) from Bogawantalava, Ceylon, well infected with a large *Opalina* of unusually irregular shape and remarkably dense ciliation (cilia lines with narrow interspaces). Nuclei mostly somewhat pointed, usually at only one end, probably a reminder of their previous division.

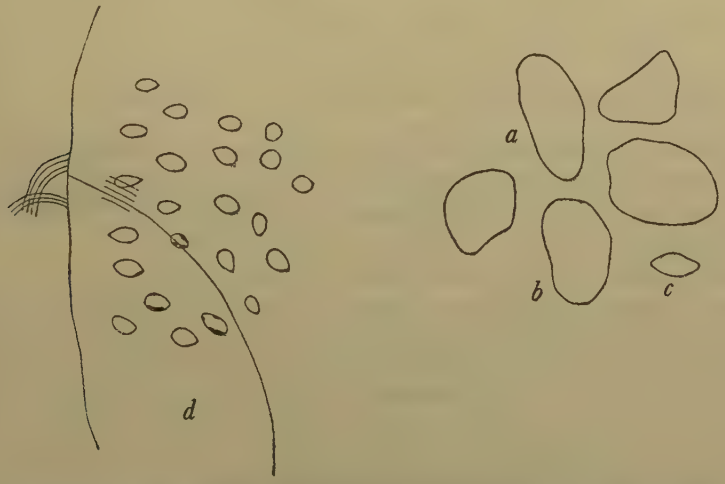

FIGURE 94.—*Opalina zeylonica*, new species, from *Polypedates eques*: *a–c*, × 78; *d*, × 673

Measurements, in microns: Body (*a*) 200 by 100, (*b*) 183 by 100, (*c*) 85 by 38, (*d*) nuclei 5.9 by 3.3, 4.5 by 3.1, length of cilia 13.8, interval between lines of cilia 1.2.

OPALINA MALAYSIAE, new species

FIGURE 95

Type: U.S.N.M. No. 22646.

Host: *Rana labialis* Boulenger.

Two specimens of this frog from Trong, Lower Siam (U.S.N.M. Nos. 24040 and 24042), each 47 mm. long, were both infected with the same species of *Opalina*. These opalinids rather closely resemble those from *R. macrodactyla* and *R. macrodon*, except that the nucleolar substance is not aggregated into one or two subcaryothecal spheroidal

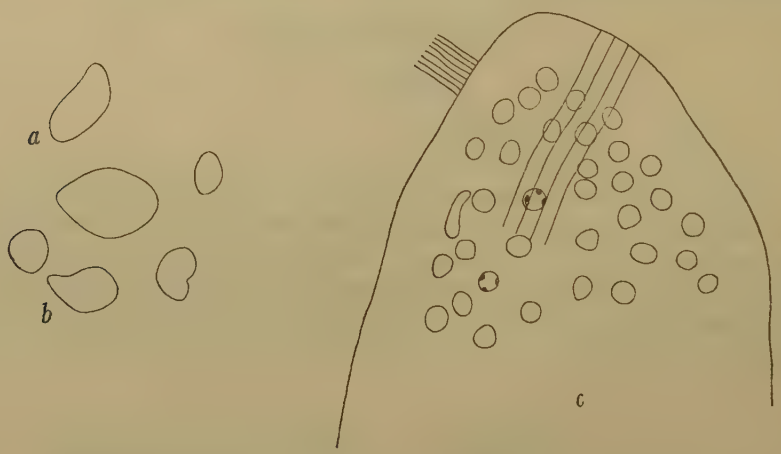

FIGURE 95.—*Opalina malaysiae*, new species, from *Rana labialis*: *a* and *b*, × 78; *c*, × 673.

disks but is in three or four or more masses as in many species. The *labialis* infections show animals half as large, or less, and they show a larger proportion of forms slender, especially behind. Their shape, too, is more irregular. They seem a distinct species.

Measurements, in microns: Body 180 by 118, 120 by 70, 67 by 42; width of body (another specimen) 81, nuclei 4.5, 3.8, 3.6, dividing nucleus 9.9 by 2.7, length of cilia 10, interval between lines of cilia 2.9.

OPALINA MALAYSIAE (?)

FIGURE 96

Host: *Microhyla ornata* Duméril and Bibron.

In a tadpole of this frog, 22 mm. long and with all four legs well developed, there was found a single *Opalina* with measurements, in microns, as follows: Body length 125, greatest width 49; length of oval nucleus 6, width 4.4; length of daughter nucleus in anaphase 6.4,

width 3.4; dividing nucleus, length 9.7, width 2.9. These are from Rangoon, Burma.

In shape and size of body and in shape and size of resting and dividing nuclei the parasite agrees with *O. malaysiae* from *Rana labialis* from Siam, but the material is scant, and the preservation does not allow detailed study and I am not definitely assigning the Opalinas to this species though probably they are the same.

OPALINA JAPONICA Sugiyama

FIGURE 97

Host: *Cacopus systoma* (Schneider).

Two specimens of this gastrophrynid were sent me from Madras, India. Both were infected with a thin *Opalina* that resembles *O.*

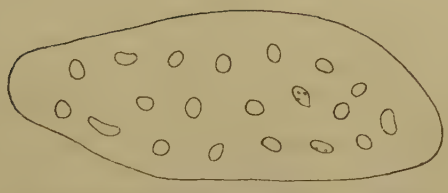

FIGURE 96.—*Opalina malaysiae* (?) from tadpole of *Microhyla ornata*, × 453.

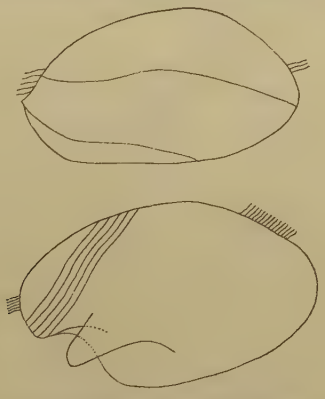

FIGURE 97.—*Opalina japonica* Sugiyama from *Cacopus systoma*, × 505.

japonica except that it is even thinner than this last species. This seems not a sufficient indication to justify even subspecific distinction. The host and the locality are new for this species.

Measurements, in microns: Body 110 by 60, 160 by 88; nuclei 3.7, 4, 4.5; length of cilia 7.2; very thin.

OPALINA JAPONICA Sugiyama

FIGURE 98

Host: *Rana limnocharis* Wiegmann, from Trihur, Cochin Province, southern India, sent by the Madras Museum. The animals of this abundant infection resemble the Java specimens, from the same frog, which I formerly doubtfully assigned to *O. japonica*. Study of this Indian infection confirms the conclusion, the posterior ends of the Japanese and the Indian forms, with their points and often exaggerated tails, forming a rather remarkable resemblance.

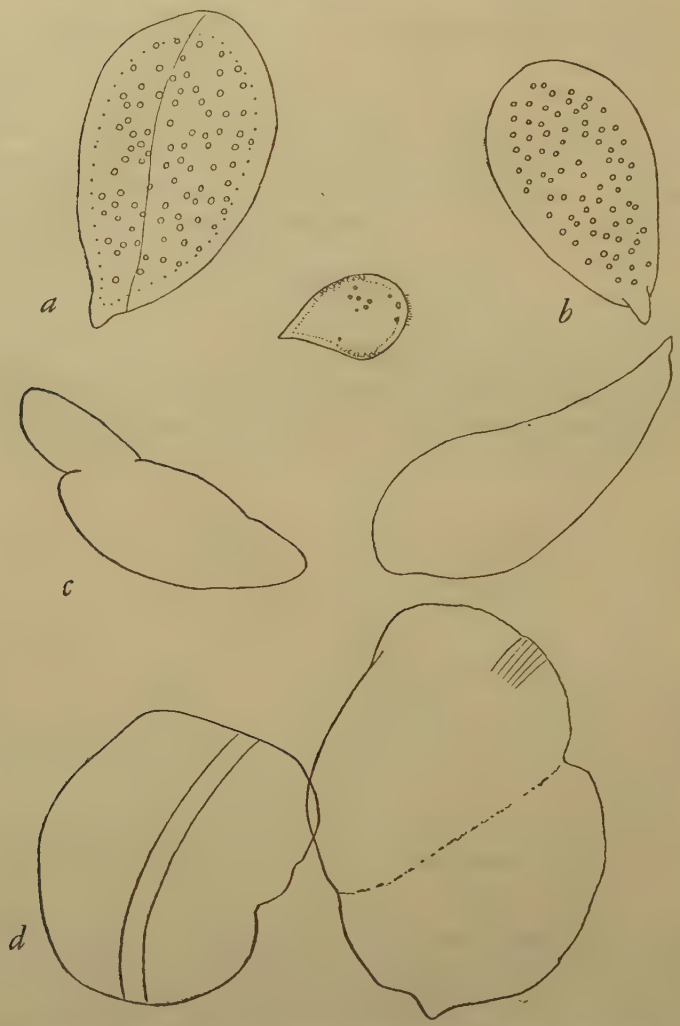

FIGURE 98.—*Opalina japonica* Sugiyama from *Rana limnocharis*, × 146 and 340.

Measurements, in microns: (*a*) body 178 by 104, cilia line interval 2.6; (*b*) body 148 by 78, nucleus 4 by 3.6; (*c*) body 158.7 by 60; (*d*) body 134 by 121.7, nuclei 4.2 by 3, 4.3 by 3.9, dividing nuclei 10 by 3.2, 15 by 4, length of cilia 8.

This is a new locality for this species but it is in the same host as before reported.

OPALINA JAPONICA JAVENSIS, new subspecies

FIGURE 99

Type: U.S.N.M. No. 22647.

Host: *Nyctixalus margaritifera* Boulenger.

A single specimen of this ranid (U.S.N.M. No. 62642), 28½ mm. long, from Mount Gade, Tjibodas, Java, was well infected with very

large Opalinas pointed posteriorly like most specimens of *O. japonica* and of about the same shapes, but much larger and with larger nuclei.

Measurements, in microns: Body 300 by 170, nuclei 4, 5, 5.4, 5.8, length of cilia 11, interval between lines of cilia 2.1.

FIGURE 99.—*Opalina japonica javensis*, new subspecies, from *Nyctixalus margaritifera: a*, × 78; *b*, × 673.

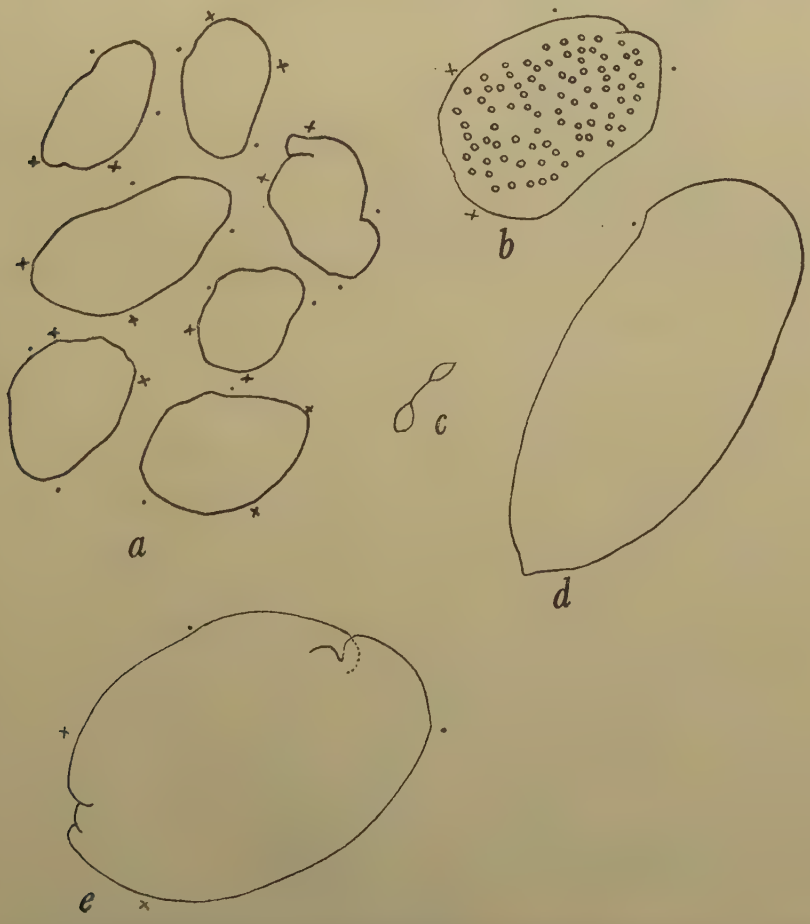

FIGURE 100.—*Opalina annandali*, new species, from *Rana tigrina: a*, A group, × 117; *b*, × 249; *c*, a dividing nucleus, × 1010; *d* and *e*, × 460. In these figures, as in some others, the limits of the anterior end of the body, right and left, are indicated each by a dot outside the body contour.

OPALINA ANNANDALI, new species

FIGURE 100

Host: *Rana tigrina* Daudin.

Of two freshly preserved specimens of this frog collected by Professor Annandale in the garden of the Indian Museum at Calcutta one bore many Opalinas of an undescribed species. The very thin posterior edge is indicated in the figures between two "x" marks. The infection shows a few individuals with abruptly sharp posterior points. Their irregular shapes are similar to those of *O. natalensis*, *O. rotunda*, and *O. zeylonica*.

Measurements, in microns: (*a*) Body 128 by 57, nuclei 3.1, 3.3, 3.8 by 2; (*b*) body 112 by 75, nuclei 3.5, 4 by 2.8.

This species resembles the *japonica* group in shape, and in dimensions of nuclei.

OPALINA CORACOIDEA Bezzenberger

FIGURE 101

Host: *Rana cyanophlyctis* Schneider.

From Tillimanti, southern India, 2 uninfected frogs; from Rhamnad, southern India, 2 frogs uninfected; from Bogawantalava, Ceylon, altitude 4,000 feet, 5 frogs, 3 infected. Bezzenberger's figure shows but one of a number of shapes. A posterior point may or may not be present. Frequently it may be exaggerated into a well-developed tail. Dividing or even fragmenting individuals (di-

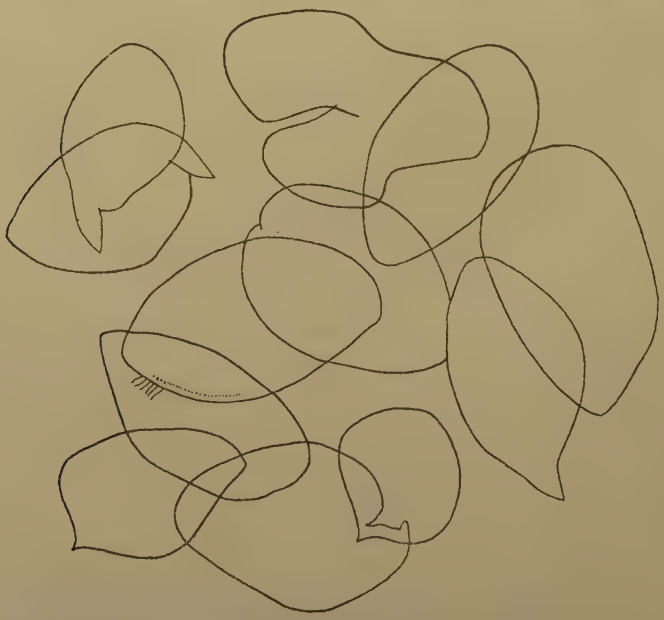

FIGURE 101.—*Opalina coracoidea* Bezzenberger, from *Rana cyanophlyctis*, × 117.

viding at the same time into more than two portions) were seen. The length of many a specimen is twice as great as in Bezzenberger's examples.

OPALINA CORACOIDEA LAHORENSIS Bhatia and Gulati

FIGURE 102

Host: *Bufo melanostictus* Schneider.

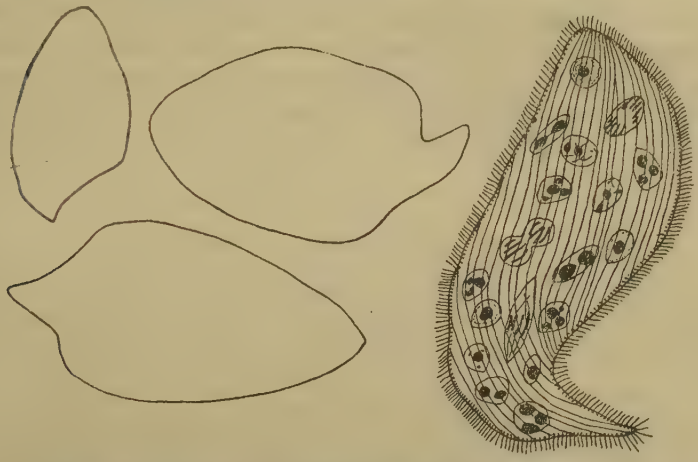

FIGURE 102.—*Opalina coracoidea lahorensis* Bhatia and Gulati from *Bufo melanostictus*. (After Bhatia and Gulati.)

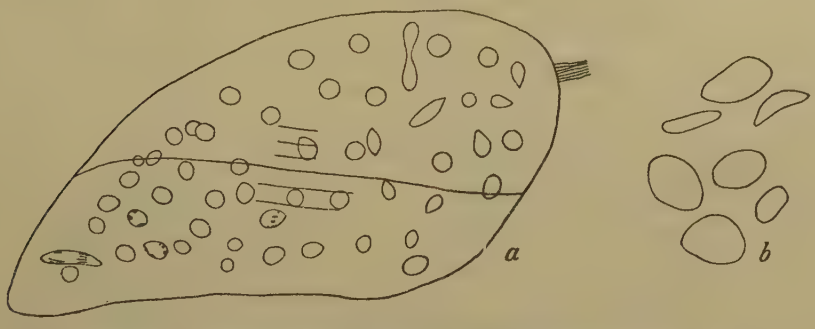

FIGURE 103.—*Opalina mantellae*, new species, from *Mantella baroni*: *a*,×673; *b*,×78.

OPALINA MANTELLAE, new species

FIGURE 103

Type: U.S.N.M. No. 22648.

Host: *Mantella baroni* Boulenger.

One specimen, 26 mm. long, from Madagascar (U.S.N.M. No. 60656), bore very abundant, small Opalinas with small nuclei. The sparse ciliation, lines of cilia rather widely spaced in front as well as in the middle of the body, is noticeable.

Measurements, in microns: Body 110 by 77, 125 by 64, 100 by 24, 70 by 26, 114 by 55; nuclei 3.3, 4, 4.2, dividing nucleus 10 by 2.2; length of cilia 6.1; interval between lines of cilia 2.8.

OPALINA species (?), probably of the JAPONICA group

FIGURE 104

Host: *Rana hexadactyla* Lesson, tadpole, a specimen from southern India in a late stage of metamorphosis, but with tail not absorbed (total length 46 mm., hind legs 19 mm. long, fore legs 6.5 mm.).

The drawings show: *a*, A *Cepedea* stage; *b*, an older *Cepedea* stage; *c*, an *Opalina*, probably not quite of mature form.

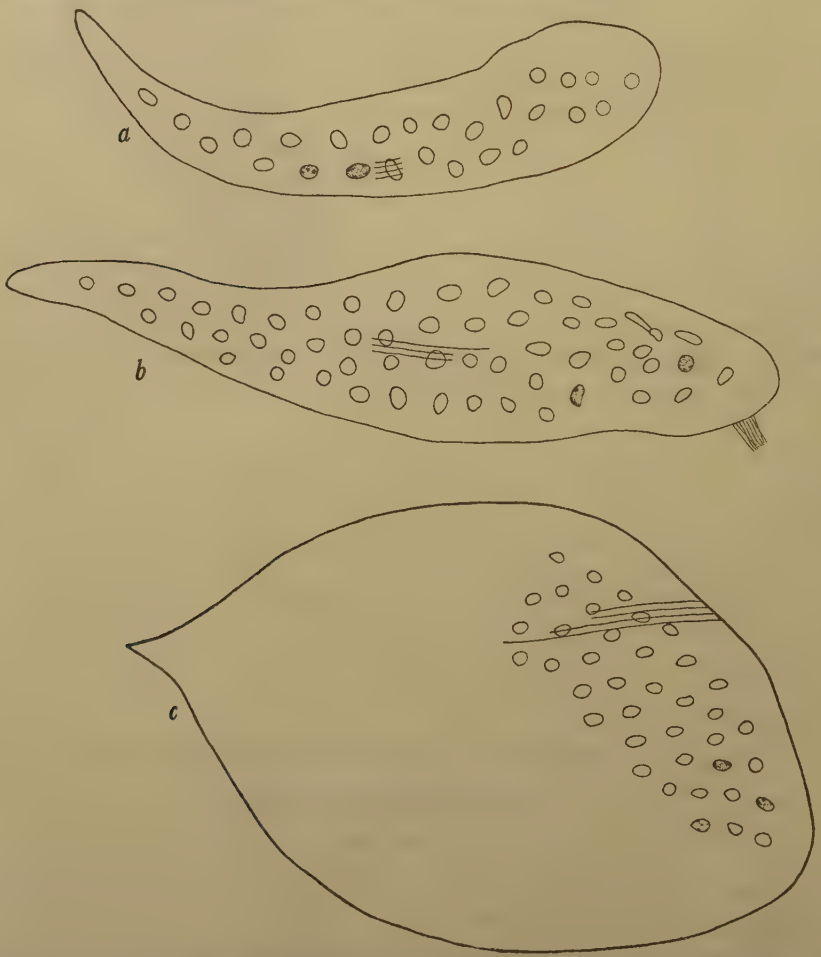

FIGURE 104.—*Opalina* sp. (?), probably of the *japonica* group, from tadpoles of *Rana hexadactyla*, × 410.

Measurements, in microns:

Measurement	*a*	*b*	*c*
Length	200	248	228
Width	36	60	170
Nucleus length	6	6	6. 7
Nucleus width	4	5. 7	4. 9
Dividing nucleus length		9. 1	
Dividing nucelus width		2. 3	
Length of cilia		10	
Interval between lines of cilia	2. 5		2. 5

In another, younger, tadpole of the same species from the same locality are larvae of apparently the same *Opalina*.

OPALINA CHATTONI Weill

FIGURE 105

Host: *Bufo melanostictus* Schneider, from Cochinchina.

This rather large *Opalina* with large nuclei is distinctively characterized, at least in the cysts and young stages, which Weill studied,

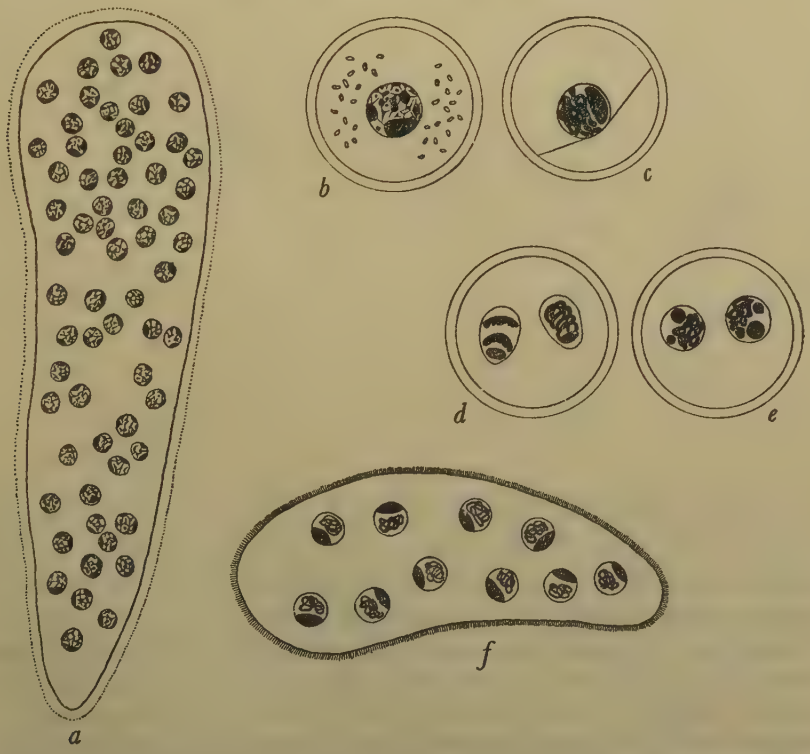

FIGURE 105.—*Opalina chattoni* Weill from *Bufo melanostictus*: *a*, Adult, × 427; *b–e*, cysts, × 1677; *f*, small individual hatched from cyst, × 1333. (After Weill.)

by having the nucleolar substance in a spherical mass and the chromatin in the form of a very well defined, rather coarse spireme. It is a very distinct species well described and well illustrated by Weill.

Measurements, in microns: Adult, body 210 by 56, nuclei 6.7, 7.4, 4.8 (daughter nucleus), length of cilia 4.3; cyst 13, with 2 nuclei 13.2, with 11 nuclei 24.6; nuclei (uninucleate) 4, (binucleate) 3.2, (4 nuclei) 3.2 by 2.4; animal just hatched from cyst, body 44.2 by 16.7, nucleus 3.2; "encysted adults," body 83, 100 by 47.

The slender form of this *Opalina* is an unusual thing for an Eastern Hemisphere species. It may be an unusually flat *Cepedea*, like *C. virgula*. There seems to be no likelihood of its being genetically related to the *Opalinae angustae* of the Western Hemisphere.

OPALINA NUCLEOLATA, new species

FIGURE 106

Type: U.S.N.M. No. 22649.

Host: *Rana chalconota* (Schlegel), from Buitenzorg, Java, three specimens.

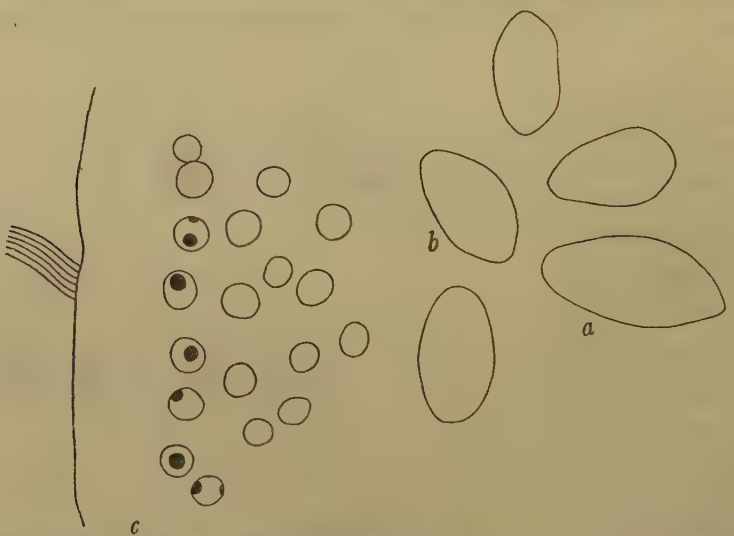

FIGURE 106.—*Opalina nucleolata*, new species, from *Rana chalconota*: *a* and *b*, × 78; *c*, × 673.

From the largest of these frogs (U.S.N.M. No. 43933), 54 mm. long, were obtained many very large Opalinas with large nuclei and of shapes rather uniform and about like those of some infections of *O. ranarum*, but their nuclei show a peculiarity that distinguishes them. Their nucleolar substance is gathered into one or two globular masses, slightly flattened against the nuclear membrane when in contact with it, apparently spherical when lying deeper in the nucleus. In my material the chromatin is scattered and not readily distinguished, not at all like the chromatin coil in young forms of Weill's

O. chattoni. Nucleolar masses, one to four in number, are found in the nuclei of many species of *Opalina*, but they are so pressed against the nuclear membrane as to be thin disks, often so thin as to escape casual observation in edge view. They are very different from these noticeable, almost spherical, bodies.

Measurements, in microns: Body (*a*) 300 by 155, (*b*) 220 by 120, (*c*) nuclei 6.9, 5.8, nucleolus up to 2.7, length of cilia 16.

OPALINA NUCLEOLATA SIAMENSIS, new subspecies

FIGURE 107

Type: U.S.N.M. No. 22650.

Host: *Rana macrodon* Duméril and Bibron.

A specimen of this host from Siam (U.S.N.M. No. 26225), 172 mm. long, bore many very large Opalinas with nuclei of only moderate size

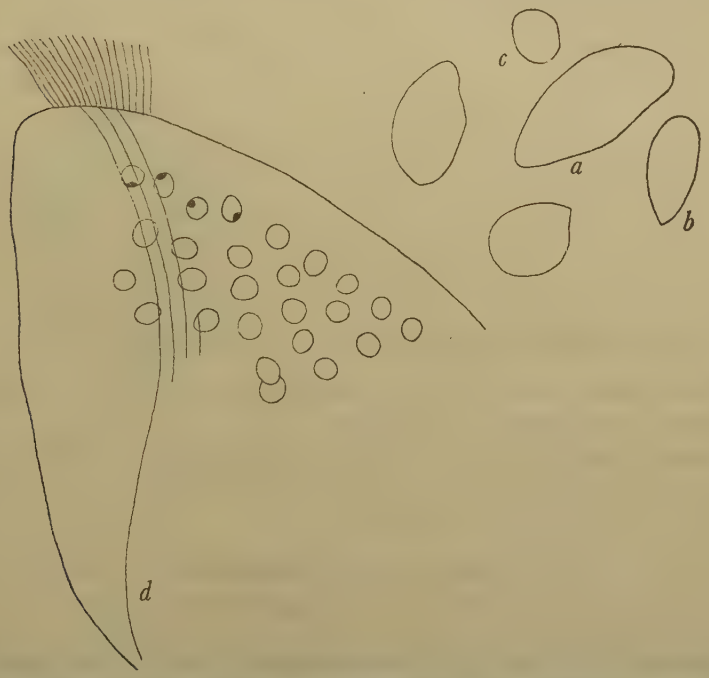

FIGURE 107.—*Opalina nucleolata siamensis*, new subspecies, from *Rana macrodon*: *a–c*, × 78; *d*, × 673.

and generally a single nucleolus in each nucleus. In size, in the range of shapes, and in the general appearance of the infection, as well as in the presence of these nucleoli, there is a resemblance to *O. nucleolata* from *Rana chalconota* from Java. The nuclei are smaller, a difference sufficient, perhaps, to justify recognition as a subspecies.

Measurements, in microns: Body (*a*) 300 by 140, (*b*) 200 by 90, (*c*) 100 by 68, (*d*) width of body 95, nuclei 4.7, 4, 4.2, dividing nucleus 5.9 by 4.9, length of cilia 10, interval between lines of cilia 2.9.

OPALINA NUCLEOLATA SIAMENSIS

FIGURE 108

Host: *Rana macrodactyla* (Günther), from Trong, Lower Siam (U.S.N.M. No. 22945), and Tonking (U.S.N.M. No. 33128), both specimens 44 mm. long.

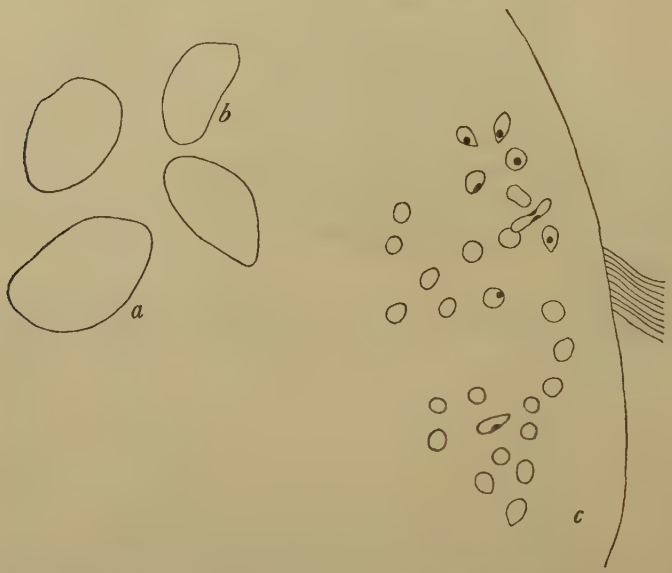

FIGURE 108.—*Opalina nucleolata siamensis* from *Rana macrodactyla: a* and *b*, × 75; *c*, × 673.

These Opalinas have still smaller nuclei than those from *R. macrodon* but in other regards resemble them.

Measurements, in microns: Body (*a*) 265 by 162, (*b*) 200 by 93, (*c*) nuclei 4.9, daughter nuclei 2.9, 3.1, dividing nuclei 4.9 by 3.3, 7 by 1.9, 9.7 by 2, length of cilia 12.8.

OPALINA OBTRIGONOIDEA Metcalf

FIGURE 109

Host: *Rana palustris* LeConte, tadpoles, from Woods Hole, Mass. Three examples of different ages, one with hind legs 6 mm. long, one with the hind legs 12 mm. long, a third having four legs and with the tail beginning to be absorbed, showed Opalinas in an *O. larvarum* stage, indicating that in this species also the narrow adults pass through a broad *Opalina* condition in their development.

Measurements, in microns: Body 120 by 96, 90 by 67, nucleus 8.7, dividing nucleus 12 by 4.5.

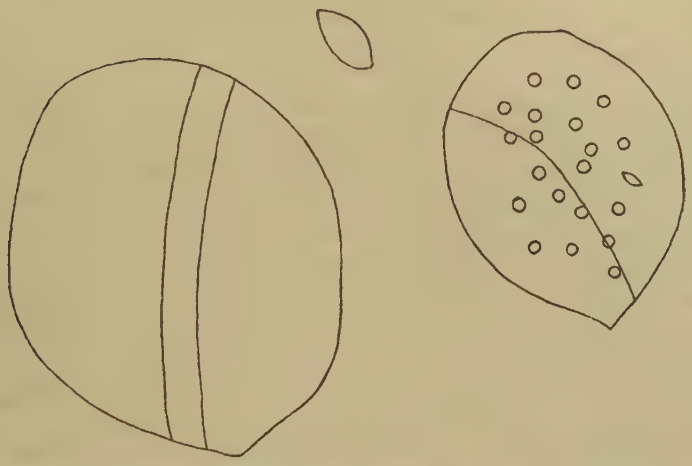

FIGURE 109.—*Opalina obtrigonoidea* Metcalf from *Rana palustris*, × 460 except the small figure, which is the outline of a nucleus in division × 1010.

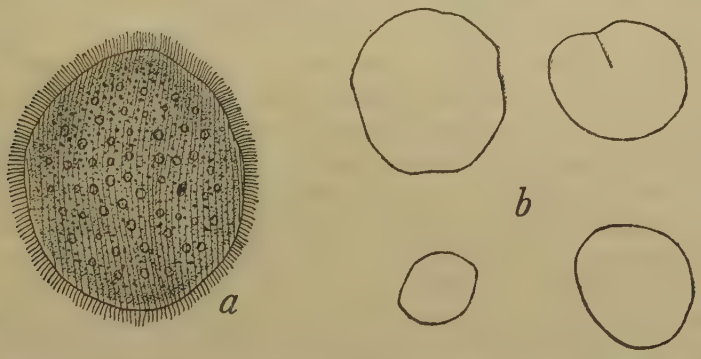

FIGURE 110.—*Opalina obtrigonoidea* forma *lata* Nie from *Kaloula borealis*: *a*, × 294; *b*, × 70.

OPALINA OBTRIGONOIDEA forma LATA Nie

FIGURE 110

Host: *Kaloula borealis* Barbour, from Nanking, China.

The body is orbicular in form and somewhat broader at the anterior end. It is almost as long as broad. The cilia are of moderate length and evenly arranged in numerous longitudinal rows. The pellicle is fairly thick and bears longitudinal grooves running throughout the length of the body. Beneath the pellicle is the layer of ectosarc, which has a vacuolar appearance.

Measurements, in microns, of a number of individuals: Body length varies from 80.6 to 245.0, width from 61.9 to 200, nucleus 4.1 to 4.2; the cilia line interval for one animal measured 3.3 anteriorly and 4.6 posteriorly.

The shape of the body of this forma is quite different from that of the type, although they are found in the same host and the size of the nuclei are nearly the same.

OPALINA LARVARUM Metcalf

FIGURES 111, 112

Opalinas of the *larvarum* type are found in tadpoles of *Rana clamitans* Latreille, *R. catesbeiana* Shaw, *R. palustris* LeConte, *R. pipiens* Schreber, *R. sylvatica* LeConte, and doubtless in the tadpoles of other frogs whose adults bear narrow species of *Opalina*. The changes in the *Opalinae* are, however, much more extensive than the final change from orbicular forms to definitely narrow *Opalinae*. I have followed the opalinids in *R. clamitans* and *R. catesbeiana* through their life history, and Miss Margaret Cowles has studied with me the life history of *O. virguloidea* in *R. sylvatica*, both tadpoles and adults.

In a paper before the National Academy of Sciences at its meeting in Washington in April 1925 (Metcalf, 1926), I reported that *O. larvarum*, after fertilization in the *R. clamitans* tadpole, started life as a uninucleate form pointed behind and resembling a *Protoopalina* with one nucleus, a condition that is found in daughter cells immediately after fission (fig. 111, *a*). The first division of the nucleus, unaccompanied by division of the body, establishes a typical *Protoopalina* condition, with two nuclei (fig. 111, *c* and *d*). Some at least of these *Protoopalina*-like young stages have a long, naked, posterior point and thus resemble adults of those species belonging to what I have regarded as the most archaic subgenus of *Protoopalina*. Nuclear division continues, occurring more often than fission, bringing about multinucleation (fig. 111). For a time, up to a condition with six to eight nuclei, the nuclei remain for the most part in a line along the longitudinal axis of the little animal and it then resembles *P. axonucleata* (fig. 111, *f*). As the nuclei become more and more numerous they no longer keep their axial alignment, assuming a *Cepedea* condition (fig. 111, *g*). Up to a stage with 10 or 12 nuclei their posterior ends are generally sharp-pointed. Those with about 20 nuclei are rounded behind. At an earlier or later stage of their development these *Cepedea*-like forms begin to broaden and flatten in front, the flattening gradually extending farther back until the cylindrical *Cepedea* is transformed into a broad, orbicular, flat *Opalina*. Some of these have as few as 8 nuclei (fig. 111, *h*). Others are found in which many nuclei are present when the flattening is beginning at the anterior end (fig. 111, *g*). In metamorphosing tadpoles with four legs, and perhaps with the tail beginning to be absorbed, some of these many-nucleate, very broad *Opalinae* become narrower and more elongated, *i. e.*, become *Opalinae angustae*. None of the broad, flat forms have been found of very large size. The opalinids all disappear at the time of metamorphosis, mostly before the narrow form is assumed.

Adult *R. clamitans* have no opalinids.[5] The tadpoles live for more than a single year in the pools. The opalinids in last year's tadpoles divide and become small, encyst and pass out of the rectum into the water, and serve as infection cysts for young tadpoles of the new crop (Brumpt, 1915). The infection is thus from tadpole to tadpole, the narrow *Opalina* stage not being found in any adults, for they are not

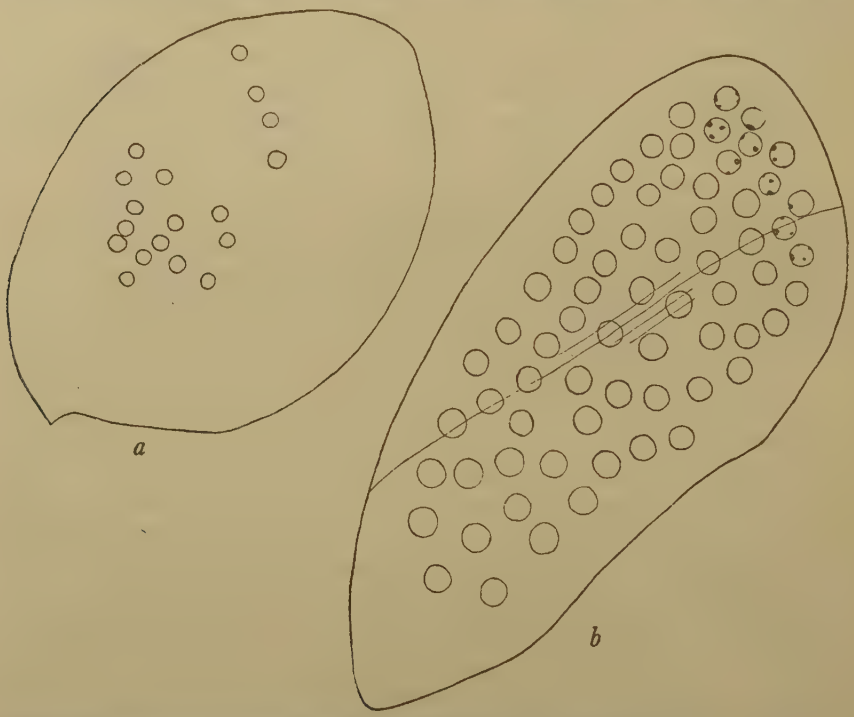

FIGURE 112.—*Opalina larvarum* Metcalf from Woods Hole, Mass. (*a*), and Philadelphia, Pa. (*b*), × 482; from a slide prepared by Prof. D. H. Wenrich.

infected, and being found only occasionally in the oldest tadpoles that have almost completed metamorphosis.

Similarly, the adult *R. catesbeiana* is uninfected, although its larvae are well infected. The large tree-frog *Hyla versicolor* shows a more delayed suppression of its *Opalinae*. I have never found a tadpole of this species uninfected and I have never found a full-grown adult infected, but small tree-frogs, less than half grown but completely past metamorphosis, show abundant narrow Opalinas of a species that I have described as *O. hylaxena*.

In tadpoles of *R. clamitans*, as I showed in 1923a, there are found many broad, flat Opalinas, generally with an abrupt, curved point

[5] There are a few reports of *Opalina* in adult *Rana clamitans* and *R. catesbeiana*. Some of these reports are of captive frogs fed on tadpoles; others are of artificial infections; a few others are of infections of adults in nature, but the infections may have been due to devouring tadpoles.

behind, named at that time *O. larvarum*. These flat, posteriorly pointed forms have 8 or more, generally less than 20, nuclei. They seem to be individuals precociously developed by flattening of "*Cepedea*-larvae" before they have lost their posteriorly pointed shape.

Brumpt (1915) desciibed retrogressive development of opalinids in the tadpoles, resulting in the formation of cysts that are capable of infecting tadpoles again. I have not traced out this process. Miss Cowles has described the course of development of *O. virguloidea* in *Rana sylvatica* (p. 556).

OPALINA (?)

FIGURE 113

In addition to undoubted *O. larvarum* from tadpoles of *R. clamitans* Latreille there are larval stages of what may be a distinct species or

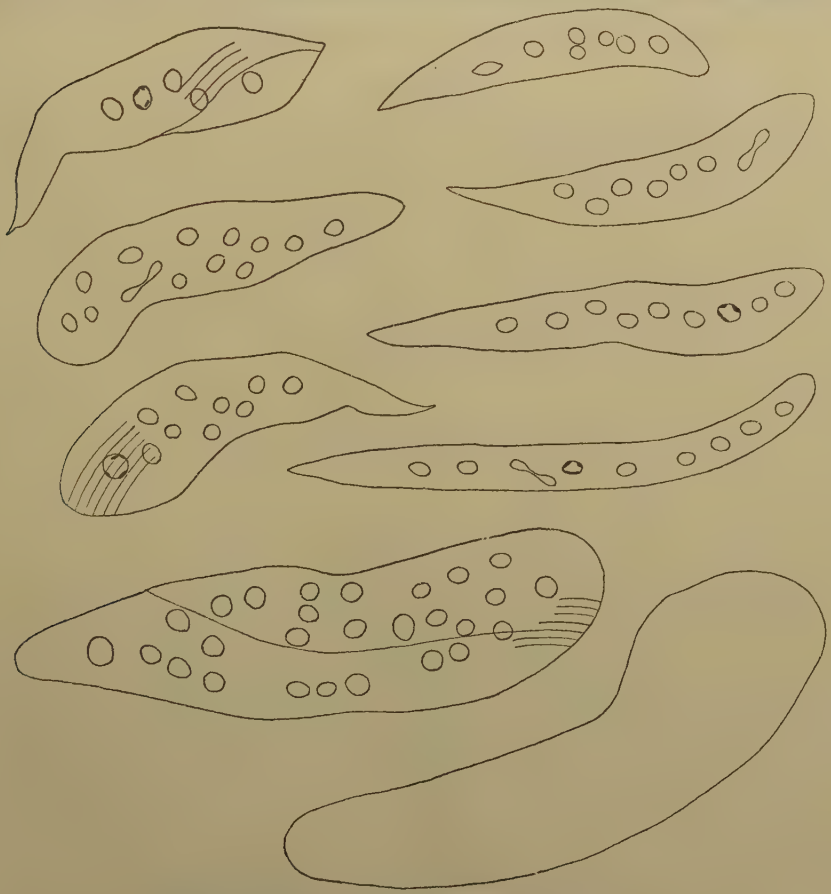

FIGURE 113.—*Opalina* (?) from tadpoles of *Rana clamitans*, × 673.

subspecies. The *Protoopalina* stages I have not distinguished from those of *O. larvarum*, although more intensive study might succeed in this. The *Cepedea* stages have smaller nuclei and narrower intervals between the lines of cilia. No broad *Opalina* stage has been seen nor any seemingly adult stage. Figure 113 shows the sorts of larval stages referred to here. It is not worth while to discuss them without further study.

In tadpoles of *R. catesbeiana* Shaw are found opalinids very similar to *O. larvarum* and showing similar developmental phenomena. The nuclei in general run smaller, and the orbicular shape, with short, usually curved, posterior point, is found less abundantly, but I am not able to find any specific or subspecific distinction between the forms in *R. catesbeiana* and those in *R. clamitans*.

The opalinids in *R. pipiens* Schreber and *R. palustris* LeConte are similar in their developmental phenomena.

OPALINA SEPTENTRIONALIS Metcalf

FIGURE 114

Host: *Hyla septentrionalis* Boulenger. Three specimens of this frog from Cuba (U.S.N.M. Nos. 7404, 7478, and 10304) and two

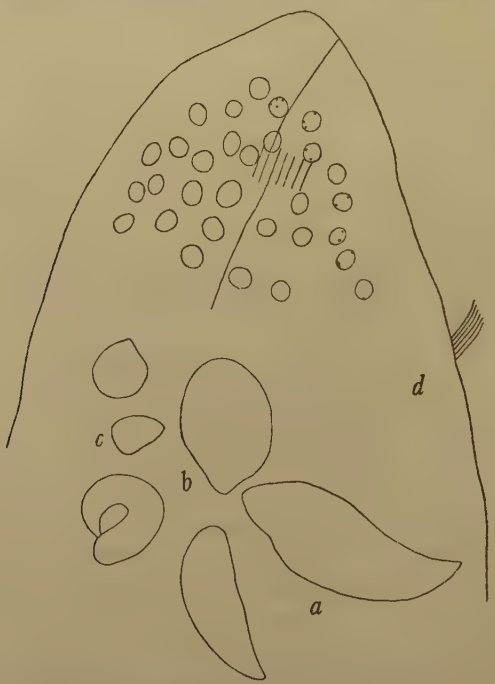

FIGURE 114.—*Opalina septentrionalis* Metcalf from *Hyla septentrionalis*: *a–c*, × 78; *d*, × 673.

out of three from Andros Island in the Bahamas (Nos. 64160 and 64163) were infected with moderately well preserved opalinas. They are evidently the same species as some seen in the same species of host in my former study, but not described because of unsatisfactory preservation.

Measurements, in microns: Body (*a*) 380 by 150, (*b*) 210 by 154, (*c*) 90 by 60, (*d*) nuclei 4.2, 4.6, 5 by 4.2, length of cilia 8.9, interval between lines of cilia 1.5. The body is large in full-sized specimens, the nuclei are small, and the lines of cilia close together.

OPALINA ELONGATA Carini

FIGURES 115, 116

Hosts: *Hyla rubra* Daudin; *H. albomarginata* (Spix).

Several years ago Prof. Adolpho Lutz, of the Oswaldo Cruz Institute, Rio de Janeiro, sent me a good slide of this interesting elongated *Opalina* from *Hyla rubra*. I have since found it in one *H. rubra*

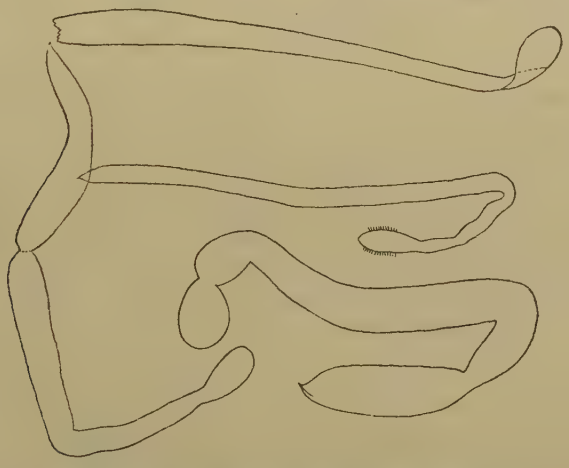

FIGURE 115.—*Opalina elongata* Carini from *Hyla rubra*, × 72.

and in six out of seven specimens of *H. albomarginata*. Especially in specimens from *H. albomarginata* they are pointed behind, often with a short spinelike point.

Measurements, in microns: From *H. rubra*, body 1,100 by 70, length of cilia 6.7; from *H. albomarginata*, body 980 by 50, 333 by 55, width of body 41.5, nuclei 7, 5.2, dividing nuclei 9 by 6, 8 by 4.5, length of cilia 6.7, interval between lines of cilia 2.

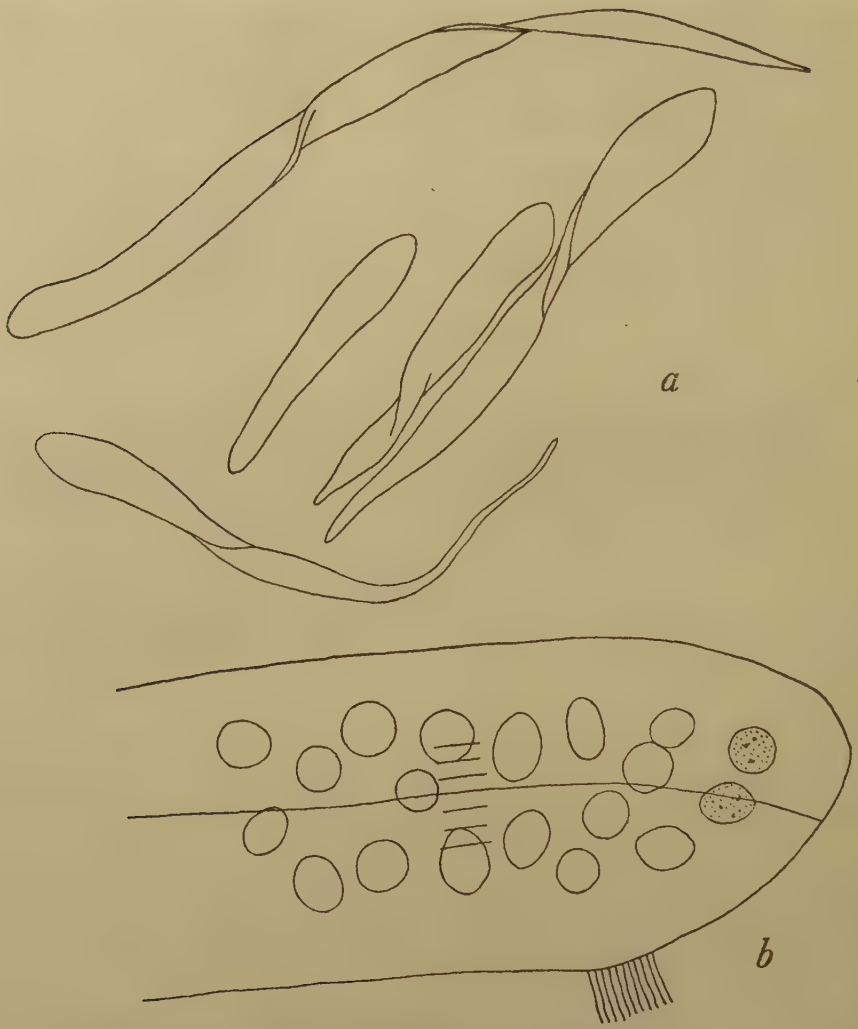

FIGURE 116.—*Opalina elongata* Carini from *Hyla albomarginata: a*, × 117; *b*, × 1010.

OPALINA CHENI Nie

FIGURE 117

Host: *Kaloula borealis* Barbour, from Nanking, China.

The body of *Opalina cheni* is roughly triangular, the base of the triangle being anterior and the apex posterior. The anterior border, however, is not straight but slightly curved, and it often inclines more to one side of the body than to the other. There are several longitudinal folds or ridges, which are gradually diminished in size as they extend from the anterior border to the posterior end. The posterior end is distinctly tapering and pointed at the extremity.

The cilia on the anterior part are much longer than the posterior ones. The pointed taillike end is deprived of cilia. The nuclei are ellipsoidal or rounded and almost of the same size. There are many spherical or elongate-oval endospherules in the endosarc.

Measurements, in microns: In a number of individuals the body length varies from 73.1 to 107.8, the width from 55.9 to 93.7, the

FIGURE 117.—*Opalina chen* Nie from *Kaloula borealis*. (After Nie.)

diameter of the nucleus varies from 4.9 by 3.6 to 7.6. The measurement of the cilia line interval at the anterior end of one animal was 3.7.

This species is characterized by the permanent presence of several folds or ridges on the surface of the body. These folds closely resemble those found in *O. obtrigonoidea* forma *plicata* Metcalf (1923a). The present species, however, differs from the latter not only in the larger diameter of the nucleus but also in its ciliation and the smaller size of the body.

OPALINA ACUMINATA Nie

FIGURE 118

Host: *Kaloula borealis* Barbour, from Nanking, China.

The form of the body varies greatly from lanceolate to ellipsoidal. The anterior edge is obliquely truncated. The posterior extremity is drawn into a short sharply pointed spinelike process. The greatest width is recorded at the region anterior to the middle of the body. The nuclei are ellipsoidal or rounded, or elongate in form during mitosis.

The layer of ectosarc is fairly thin, but it is well marked off from the endosarc, which is vacuolated in appearance.

Measurements, in microns: In a number of specimens the length of the body varies from 59.5 to 131.6, the width from 29.7 to 56.0, the diameter of the nucleus from 5.3 to 5.8. The cilia-line interval on one animal measured 1.9 on the anterior end and 2.1 on the posterior end.

The number of nuclei in the majority of the individuals of *O. acuminata* has been estimated from 6 to 36, and they are arranged in the outer layer of the endosarc.

At a glance, *O. acuminata* resembles Metcalf's *O. carolinensis* (1923a) because of similarity in the shape of the body. The latter

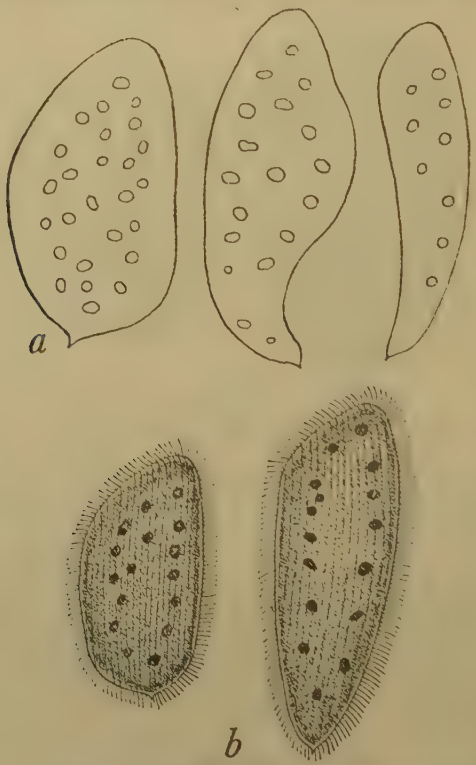

FIGURE 118.—*Opalina acuminata* Nie from *Kaloula borealis*: *a*, Three individuals showing range of size and form; *b*, two individuals showing structure. All × 340. (After Nie.)

differs from the present species in its larger size (400μ in length, 110μ in width), shorter cilia, thicker ectosarc, and smaller nuclei.

The abruptly acuminated posterior end serves to distinguish the species from *O. obtrigonoidea*.

OPALINA UNDULATA Nie

FIGURE 119

Host: *Rana limnocharis* Wiegmann, from Nanking, China.

The body is elongated and corkscrew-shaped. The anterior half is considerably broadened and flattened, while the posterior half is greatly narrowed and twisted or spirally plicated into three "undu-

lating processes." It appears slightly truncated or rounded at the anterior end, and tapers gradually along the "undulating processes" toward the pointed posterior end. The length of the body is about 4 times the greatest width.

Cilia bordering the anterior part are slightly longer than those found at the posterior region. The ectosarc, which has a vacuolated appearance, is rather thick as compared with that of the other species of *Opalina*. In addition to the nuclei, there are many small spherical or elongate-oval endospherules in the endosarc.

Opalina undulata is quite different from *O. spiralis* Metcalf (1923a) in its plicated manner of the posterior half of the body. In the

FIGURE 119.—*Opalina undulata* Nie from *Rana limnocharis*, × ca. 300. (After Nie.)

latter species the spirals are rather irregular in shape and number and it shows no "undulating processes" as observed in the present species. The broader anterior portion (130–140μ) and the smaller size of the nuclei (4.3–4.7μ) also serve to distinguish *O. spiralis* from *O. undulata*.

Measurements, in microns: The body length varies from 182 to 378, the width from 44.8 to 105; the average diameter of the nuclei is 5.7, and the average diameter of endospherules is 1.5.

OPALINA FABER Carini

FIGURE 120

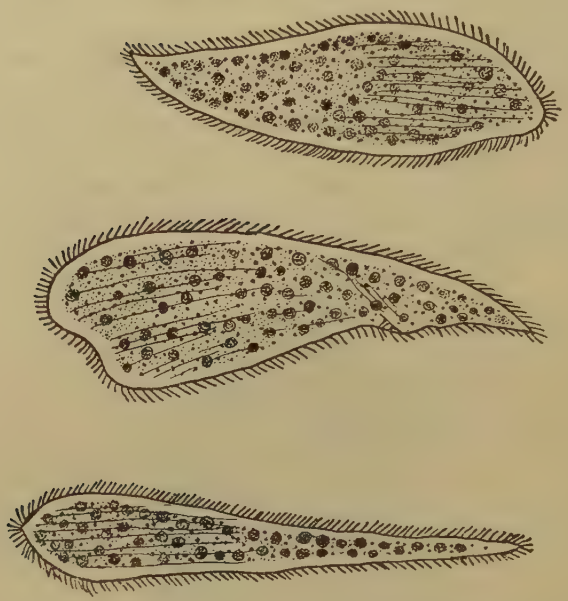

FIGURE 120.—*Opalina faber* Carini from *Hyla faber*, × ca. 230. (After Carini.)

Host: *Hyla faber* Wied, from Brazil.

The body is flat, elongated, and slightly bent. There is a great range of variation in size. Most of the fully grown individuals measure 150μ to 200μ in length, and the largest specimens measure 300μ. Some slender individuals measure 30μ to 40μ in width; the larger ones 60μ to 70μ or more.

The large specimens are fusiform, with maximum width in the middle; the posterior end generally tapers. In the large forms there are as many as 7 to 9 rows of spherical nuclei. Figure 120 shows three medium-sized specimens; a long and slender one measuring 285 by 44μ, a large and fusiform one measuring 228 by 80μ, and one showing the beginning of longitudinal division.

OPALINA NEBULOSA Carini

FIGURE 121

Host: *Hyla nebulosa* Spix, from Brazil.

The body is elongated, slightly bent, and comma-shaped. The maximum width is near the anterior end; the body becomes slenderer toward the posterior end, which is blunt. The contour of the anterior end is not round but appears as if cut obliquely on one side.

Measurements, in microns: The individuals that appear fully grown average 200 to 250 in length, but some measure 300. The width is 40 to 50, but there are some large forms that measure up to 70 and 80. There is frequently seen a border of clear ectoplasm. In the cytoplasm there are a number of spherical nuclei 3.5 to 5 in diameter, irregularly distributed in 4 to 6 longitudinal rows.

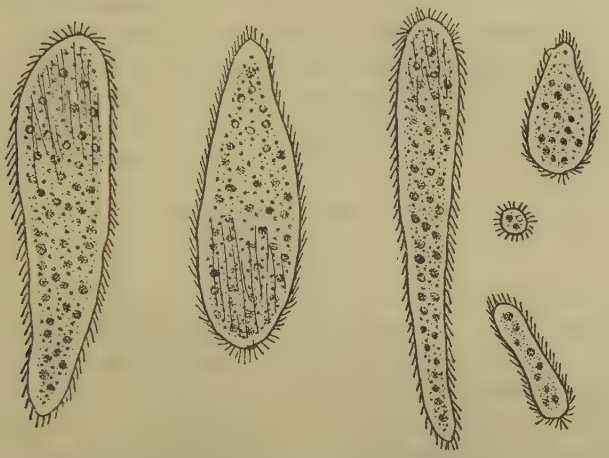

FIGURE 121.—*Opalina nebulosa* Carini from *Hyla nebulosa*, × ca. 200. (After Carini.)

Three fully grown animals and three smaller forms are shown in figure 121.

OPALINA RADDIANA Carini

Host: *Hyla raddiana* Fitzinger, from Brazil.

The body is greatly flattened, often bent.

Measurements, in microns: The average length is 300 to 500, but there are individuals that measure 450. The slender forms have a width of 40; most individuals are about 60 wide, the largest specimens measuring 80. The nuclei are spherical, 5 to 6 in diameter. The endospherules are large and numerous. The pellicle is thin. (Carini gives no drawings. There is insufficient description.)

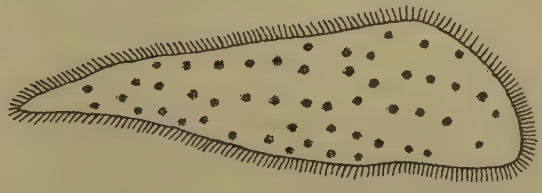

FIGURE 122.—"*Opalina sudafricana*" Fantham from *Bufo regularis*, × 300. (After Fantham.)

"OPALINA SUDAFRICANA" Fantham

FIGURE 122

Host: *Bufo regularis* Reuss.

Fantham's drawing and measurements, in microns, are given: Body length 106 to 287.5, width 25 to 87.

This seems either a narrow *Opalina* or an unusually flat *Cepedea*. Narrow Opalinas are not known from the Eastern Hemisphere except for less than half a dozen doubtful cases and except for *O. obtrigona*, which, with its hylid host, is a late immigrant from North America. But Fantham's drawing appears more that of an *Opalina* than a *Cepedea*. Restudy of fresh material would probably be worth while.

OPALINA TRIANGULARIS Ghosh

FIGURE 123

Host: *Bufo melanostictus* Schneider.

Ghosh's description (1918a), insufficient for certain identification, is as follows: "Body flattened, leaf-like, twice as long as broad or less, widest in the anterior body half; one side nearly straight, and the other strongly convex giving the appearance of two curved sides meeting at the widest part of the body; anterior end rounded and in the same line with the straight side; posterior end tapering and rounded; numerous nuclei. In the lower part of the intestine and upper part of the rectum of *Bufo melanostictus*."

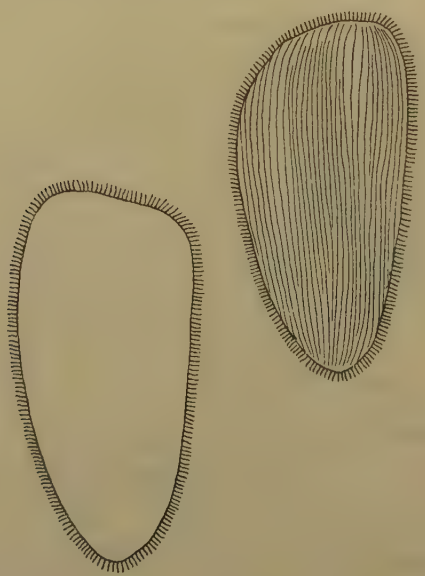

FIGURE 123.—*Opalina triangularis* Ghosh from *Bufo melanostictus*.

OPALINA species (?)

In a specimen of *Rana jerboa* Günther (U.S.N.M. No. 44013) from Tjiburrum, Java, Mount Gede, at 6,400 feet altitude, one specimen only of a large *Opalina* was found. It was not preserved.

OPALINA species (?)

Two examples of *Rana verrucosa* Gravenhorst, 32 and 36 mm. long, collected in September 1914 and sent by the Madras Museum from Parambikulam, Cochin State, southern India, showed a few remnants of a large *Opalina*, too poorly preserved for study.

"OPALINA TERMITIS" de Mello

FIGURE 124

Hosts: Two termites—*Leucotermes indicola* from India and *Calop-termes militaris* (?) from Daman, Portuguese India.

This form is somewhat like *Opalina*, yet one can but doubt its belonging to the Opalinidae. I have found only de Mello's reference

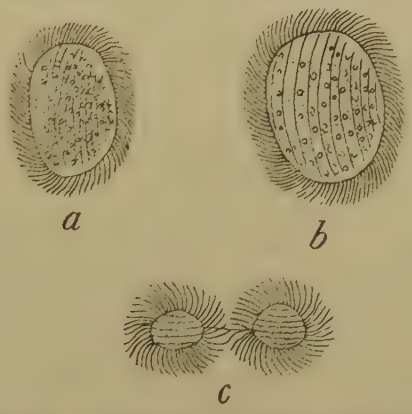

FIGURE 124.—*"Opalina termitis"* de Mello. (After de Mello.)

to the form in the Report of the Third Entomological Meeting at Pusa, India, and this and the accompanying figures (copied in our fig. 124) are insufficient for critical determination. Especially figure 124, *c*, a stage in what must, from the course of the lines of cilia, be a transverse division, is unlike any appearance hitherto found in a dividing *Opalina*. One cannot tell whether the animal is flat or spheroidal. Another major difficulty in classing this as an *Opalina* is the great difference between the aquatic reproductive habits of the Anura and the terrestrial reproduction of the termites. The life history of the opalinids includes encystment of minute individuals, their passing into pools of water where they lie upon the bottom until they are ingested by browsing, vegetarian, aquatic larvae of Anura in whose recta they hatch from the cysts and develop into male and

female gametes, which fuse and grow to adult opalinids. One can imagine possible infection of a termite from cysts deposited by an anuran with its feces in, say, a hollow log, and the continued infection of termite from termite in the new environment by means of cysts in the feces. But before this is accepted it must be much better indicated than it now is, the presence in two termites of forms doubtfully resembling *Opalina* being all that we have to go upon.

THE GROWTH OF OPALINA VIRGULOIDEA METCALF IN THE TADPOLES OF RANA SYLVATICA LECONTE

By MARGARET COWLES and MAYNARD M. METCALF

The *Opalina* of the American wood frog was named *virguloidea* by Metcalf (1923a). In the same publication he described a species, *O. larvarum*, from the common green frog (*Rana clamitans*) and suggested that this very broad, orbicular opalinid belonged to the subgeneric group *Opalinae angustae* rather than to the *Opalinae latae*, this proposed classification based on the presence of a minute curved posterior point, now known to be sometimes found also in broad Opalinas. At that time late stages in the life history had not been seen. Material secured later showed that this guess, though based on a mistaken assumption, was correct, for the tadpoles ready for metamorphosis, with four legs present, though with the tail not yet absorbed, show Opalinas of narrow form.

On the basis of observations upon the development of *Opalina larvarum* in the tadpoles of *Rana clamitans* and of a similar species in the tadpoles of *Rana catesbeiana*, Metcalf reported in 1925 to the National Academy of Sciences that the *Opalinae angustae* pass through a series of larval stages recapitulating the evolution of the family Opalinidae. The *Opalinae angustae* are the most highly evolved members of the family, and it was found that their larval stages are, first, *Protoopalina*-like, then *Cepedea*-like, that then the *Cepedea* larvae flattened, first in front and then throughout the length of the body, becoming broad *Opalinae*, and that before metamorphosis these broad *Opalinae* changed to narrow forms. No drawings were published with this report, but charts of the phenomena in *Opalina larvarum* were shown to the American Society of Zoologists in Cleveland, Ohio, during the Christmas holidays in 1930. It was also reported that *Zelleriella* passes through a *Protoopalina* stage, *Cepedea* through a *Protoopalina* stage, the *Opalinae latae* through a *Protoopalina* stage followed by a *Cepedea* stage, and the *Opalinae angustae* through three larval stages, representing, first, *Protoopalina*, then *Cepedea*, then the *Opalinae latae*, finally assuming their definitive narrow form. It has seemed worth while to review here this remarkable life history for another narrow species of the genus *Opalina* and to give drawings of the larval stages in their sequence.

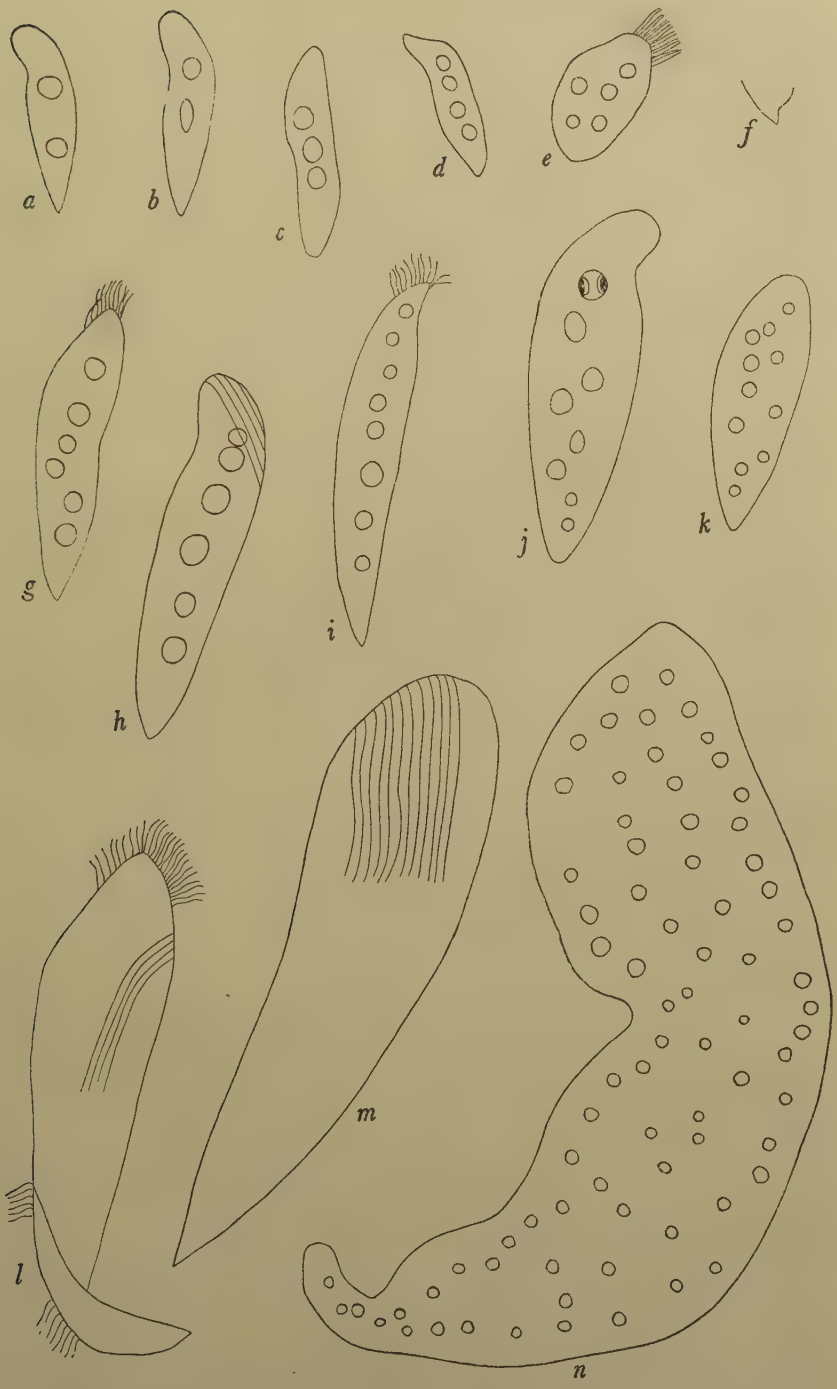

FIGURE 125.—*Opalina virguloidea* Metcalf: Development in the tadpoles of *Rana sylvatica*, × 505.

The drawings here published (fig. 125) are mostly self-explanatory and little description is needed. Figure 125, *a*, is a typically *Protoopalina*-like larva; *b* shows the posterior nucleus entering mitosis; *c* and *d* show stages with three and four nuclei, becoming *Cepedea*-like larvae; *e* and *f* depict individuals that have respectively five and six nuclei and that are much flattened and represent the *Opalinae latae* larvae, such as those in *O. larvarum* that are much more emphasized. In tadpoles of *O. virguloidea*, the broad stage is very brief and occurs in younger larvae with fewer nuclei. It seems quite possible that in some narrow species of *Opalina* it may be found to be wholly suppressed. Indeed it may perhaps be omitted from the life history of some individuals of *O. virguloidea*, for larvae in this stage are less numerous in our slides than *Cepedea* forms with five to eight nuclei.

Figure 125, *h* and *i*, resemble *P. axonucleata*. Larvae of this type, with about 6 to 10 nuclei arranged along the midline of the body, are very numerous and probably occur in the growth of every individual. As they get older and the nuclei increase in number, the linear arrangement of the nuclei is lost (fig. 125, *j* and *k*). By further multiplication of nuclei, by growth and elongation, and by flattening, first at the anterior end, then throughout the body, larvae resembling *O. obtrigona* arise (fig. 125, *m*). Comma-shaped forms with marked curvature, especially of the anterior end, then arise, giving the adult form (fig. 125, *n*), the further changes being increase in size and increase in number of nuclei.

The recapitulation of the phylogeny is remarkably complete, but the broadening of the body occurs in this species earlier than in *O. larvarum*. The trend toward increase in number of the nuclei and the trend toward flattening and broadening of the body are both present in both *O. virguloidea* and *O. larvarum*, but the emphasis upon broadening and flattening comes relatively a little earlier in the former species.

DIAGNOSTIC CHARACTERS IN OPALINIDAE

There is much divergence in form of the body between different genera, different species, and even different individuals of the same species in the Opalinidae, but still there is a schematic plan upon which they are built. *Protoopalina* is a spindle with a broad, rounded, anterior end and a more tapering posterior part of the body. The anterior end is bent and sometimes flattened; the posterior end may have a long, slender point, or a much more abrupt point, or may be rounded showing no point at all. There is much individual variation, and this is more marked in preserved specimens, which may have been killed in any of the diverse forms that the flexible bodies may assume in life. For example, the bend in the anterior part of the body may not appear.

But the variation in form is more than a matter of a flexible body assuming temporarily different shapes. This may account for only a small part of the variation. At the posterior end of the body there may be, for example, a slender, tapering, naked "tail," like a whiplash. In some species this seems to be always seen (fig. 21, *Protoopalina*, subgenus I) (cf. also figs. 12, 13, and 36 in Metcalf, 1923a). In other species it appears in some individuals but not in others. Compare *P. caudata*, in which, in the same individual host may sometimes be seen individuals with slender tails and others with rounded posterior ends (see fig. 19 in Metcalf, 1923a). Some infections of *P. caudata* may contain only posteriorly rounded individuals; other infections contain mostly tailed forms with some rounded ones; still others may show mostly rounded ones and a few with short, curved, posterior hook, either sharp or with a blunt point. These are not temporary conditions assumed by any individual at will, but are constant and characteristic for the individual. Furthermore, there are found, in addition to the comparatively slender individuals, others that are very broad and swollen (see fig. 17, *a*, in Metcalf, 1923a). One does not know how to regard the great divergence found. Is it an indication of races within the species? It may be. Probably only breeding experiments would solve the puzzles, and the peculiar parasitic habits make such experiments forbiddingly difficult. Another variety of posterior end, with a short spine, is found in *P. stevensoni* (Metcalf, 1923a, fig. 26), and this appears also, by the way, in *Cepedea spinifera* (Metcalf, 1923a, fig. 104).

Cepedea has the bent spindle form of its immediate ancestor *Protoopalina*, but we do not find the markedly divergent types of posterior end. We do, however, find some species with two types of body, one rather slender, the other stocky. Whenever a species of *Cepedea* or of *Protoopalina* shows these two types, intermediate forms in the same species, and usually in the same infections, will be found, the same individual host containing all three—slender, broad, and intermediate. More or less faint indications of a posterior hook or spine appear in some Cepedeas, for example, in *C. dimidiata* forma *zelleri* (see Metcalf, 1923a, fig. 105, middle, left-hand drawing). In some Opalinas a posterior spine or faintly indicated hook may appear, and in some Zelleriellas the asymmetrical, posterior hook may be greatly developed, for example, in *Z. antunesi* (fig. 42); and in the degree of development of any of these features individual opalinids as well as species differ.

This all makes taxonomic conclusions uncertain until after study of much material from many hosts. Without such intensive study, specific diagnosis must be tentative.

The two flattened genera, *Zelleriella* and *Opalina*, each show in many individuals of many species indication of a bend in the anterior

part of the body, and a posterior point, or spine, or hook, either curved or straight, but not a tail, is seen (figs. 98, 99, 100, *d*). In some Opalinas, as also in some Cepedeas, it is evident that the posterior spine or hook is situated a little to one side of the posterior tip of the body. While no adult *Cepedea* or *Opalina* has been found with a slender, tapering tail, this is their regular condition in their early larval history.

One who is familiar with the family as a whole cannot but feel that the same schematic form underlies them all, though what are adult features in *Protoopalina* may appear only in the larvae of the multinucleate genera.

What has just been written shows that one must be cautious in evaluating conditions of form of body for purposes of taxonomic description. He must be cautious also in the use of other characters. There is much diversity in actual size of nuclei at different stages of the life history. In general the nuclei decrease in size as they increase in number during development in the multinucleate genera, but, perhaps, as Hegner and Wu (1921) have claimed, there is for each species a rather constant ratio between size of nucleus and the bulk of cytoplasm over which each nucleus presides. Whether within a species there are races that differ in the nucleocytoplasmic bulk relations is not determined.

Nuclear conditions, especially the chromosomes, their number, sizes, and forms, when carefully studied, can be relied upon for specific diagnosis. The mitotic phase of the nuclei at the time when division of the body begins might be constant and is possibly a usable character in the binucleate genera, especially in *Zelleriella*, whose species are, in many cases, so similar that any diagnostic possibility is eagerly seized upon. The interval between lines of cilia, if we remember the habit of interpolating secondary lines between the primary in the anterior part of the body, appears to be a more constant character than one might have anticipated, and there are a few cases of species with cilia unusually closely set in the lines. If one is careful to compare corresponding portions of the body, these apparently quite constant characters are useful.

All this emphasizes the fact that one must study much material from different sources and study it with minute attention, and that even after such extensive and minute study there will still be uncertainty because breeding experiments cannot be made to enable one to differentiate between species and intraspecific races. There is great probability that extreme races in one species may overlap extreme races in another species. After studying the Opalinidae for 25 years I am increasingly hesitant about positive specific diagnosis in numerous cases. In others, on the other hand, one has no such hesitation. The Opalinidae are an exasperating group taxonomically to one

whose purpose is to place each species in its clearly distinct pigeonhole, but it is an instructive group. We have discussed the effect of the internal habitat in removing much of the struggle for existence and allowing all types that do appear to persist. This is very likely one of the chief keys to the conditions of speciation found in the family.

Mention might be made of the rotating, spiral progress of a swimming opalinid of any species, similar to the manner of swimming of euciliates. This pattern of swimming path in the Opalinidae seems to be influenced by several structural features: (1) The bend in the anterior portion of the body, (2) the insertion of the cilia in spiral lines, (3) the greater number and length of the cilia in front than behind. These all cooperate to produce rotation, progress, and the swinging of the anterior end of the body through a wider arc than that described by the posterior end. Note that the spiral position of the oral groove and its emphasized cilia in the euciliates cooperate in them to emphasize the spiral, rotating progress, but the mouthless, grooveless opalinids have the same pattern of motion. It is a fundamental feature, and an ancestral feature, seen in Flagellata, Protociliata, and Euciliata.

In many euciliates with an anterior bend in the body the oral groove and mouth is in the region of the bend. One gets the impression that the mouth of the ancestors of the Opalinidae may likely have been near the bend. Detailed study of the arrangement of the "neuromuscular" fibrillae would be worth while, to see if there is any asymmetry or any "center" in this region to make this interpretation probable.

In a previous publication (Metcalf, 1923a) I gave a table of the hosts of the known Opalinidae and of the geographic occurrence of the opalinids. The following table presents the new data since that publication.

166877—40——7

Species of opalinid	Host species	Family of host	Known geographic occurrence of the opalinid in the host named	Known geographic occurrence of host	Known geographic occurrence of genus of host
Protoopalina appendiculata Fantham.	*Rana fuscigula* Duméril and Bibron.	Ranidae.	Johannesburg, South Africa.	Western and southern Africa.	Cosmopolitan, except absent from Australia (one species in the northernmost tip), New Zealand, Tasmania, South America (except one species in northernmost parts), and oceanic islands.
bibronii, new	*Pseudophryne bibronii* Günther.	Bufonidae.	Southeastern Australia.	Australia.	Australia, Tasmania.
borneonensis, new	*Polypedates reinwardtii* (Boie).	Ranidae.	Borneo.	Sumatra, Borneo, Java.	Madagascar, Ceylon, India, China, Japan, Philippines, East Indies.
caccosterni Fantham	*Cacosternum boettgeri* (Boulenger).	Microhylidae.	Johannesburg, South Africa.	Kaffraria, Uganda.	South Africa.
[*capensis*, new]	*Heleophryne regis* Hewitt.	Leptodactylidae.	Cape Province, South Africa.	Cape Province, South Africa.	Southern and western Africa.
caudata microhyla Nie	*Microhyla ornata* Duméril and Bibron.	Microhylidae.	Southern India.	Southern China, Formosa, Tonkin, Hainan, Burma, Assam, Siam, Annam, Malay Peninsula, India, Ceylon.	China, Ceylon, various parts of East Indies, Sumatra, Java, Borneo.
luzonensis, new	*Kaloula picta* Eydoux and Souleyet.	Do.	Luzon, Philippine Islands.	Philippine Islands.	India, southeastern Asia, Philippines, East Indies.
meridionalis Fantham and Robertson.	*Rana delalandii* (Tschudi).	Ranidae.	Johannesburg, South Africa.	Southern and eastern Africa.	Cosmopolitan, except absent from Australia (one species in northernmost tip), Tasmania, New Zealand, South America (except one species in northernmost parts), and oceanic islands.
nyanza Lavier	*Varanus niloticus* Linnaeus.	Varanidae.	Shores of Lake Victoria Nyanza.		
octomixa Fantham	*Bufo carens* A. Smith.	Bufonidae.	Johannesburg, South Africa.	Eastern and southern Africa.	Cosmopolitan, except Australia, Madagascar, the Seychelles.
ovalis Fantham	*Rana fuscigula* Duméril and Bibron.	Ranidae.	Do.	Western and southern Africa.	Cosmopolitan, except absent from Australia (one species in northernmost tip), Tasmania, New Zealand, South America (one species in northernmost parts), and oceanic islands.

stejnegeri Metcalf	*Ascaphus truei* Stejneger.	Discoglossidae.	Olympic and Siskiyou Mountains, extreme northwestern United States of America.	Western Washington, northwestern California.	Only one species known.
transvaalensis Fantham	*Bufo regularis* Reuss.	Bufonidae.	Johannesburg, South Africa.	Africa south of Sahara Desert.	Cosmopolitan, except Australasia, Madagascar, the Seychelles.
xamachana, new	*Eleutherodactylus luteolus* (Gosse).	Leptodactylidae.	Jamaica, West Indies.	Jamaica, West Indies.	Tropical America.
xenopodos Metcalf	*Xenopus laevis* (Daudin).	Pipidae.	Johannesburg, South Africa.	Southern Africa.	Central and southern Africa.
yunnanensis, new	*Bombina maxima* (Boulenger).	Discoglossidae.	Yunnan, China.	Eastern Himalayan highlands.	Europe, north to the Baltic coast, to (not into) Russia, Korea, northeastern and southwestern China.
Zelleriella antunesi Pessôa	*Bufo crucifer* Wied.	Bufonidae.	Rio de Janeiro, Brazil; Montevideo, Uruguay.		Cosmopolitan (except Australasia, Madagascar, the Seychelles).
Do	*Bufo d'orbignyi* Duméril and Bibron.	Do.	Brazil, Uruguay.	Brazil, Uruguay.	Do.
Do	*Leptodactylus ocellatus* (Linnaeus).	Leptodactylidae.	Rio de Janeiro, Brazil; Montevideo, Uruguay.	Tropical America from Central America to Buenos Aires, West Indies.	Tropical America from Central America to Buenos Aires, West Indies.
brasiliensis (Pinto)	Do.	Do.	Do.	Do.	Do.
Do	*Crossodactylus gaudichaudii* Duméril and Bibron.	Do.	Rio de Janeiro, Brazil.	Rio de Janeiro, Upper Amazons.	Brazil.
dubia, new	*Eupemphix nana* Boulenger.	Bufonidae.	Angra dos Reis, State of Rio de Janeiro, Brazil.		
[of Eleutherodactylus miliaris].	*Eleutherodactylus miliaris* (Spix).	Leptodactylidae.	Do.	Brazil.	Tropical America.
ovonucleata, new	*Leptodactylus pentadactylus* (Laurenti).	Do.	Belle Horizonte, Brazil.	Lesser Antilles, Costa Rica, Tropical America.	Tropical America from Central America to Buenos Aires, West Indies.
ovonucleata bufonis, new	*Bufo sternostignatus* Günther.	Bufonidae.	Tehuantepec, Mexico.	Southern Mexico into northern South America.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
piscicola da Cunha and Penido.	A catfish (?). Name not given.	Teleost fish.	Paraguay River.		
trinitatis Metcalf	*Elosia lateristrigata* Baumann (?).	Leptodactylidae.	Angra dos Reis, State of Rio de Janeiro, Brazil.		Tropical America.
uruguayensis, new	*Bufo arenarum* Hensel.	Bufonidae.	Montevideo, Uruguay.	Southern Brazil to Buenos Aires.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
uruguayensis form *quadrata*, new.	*Bufo dorbignyi* Duméril and Bibron.	Do.	Brazil, Uruguay.	Brazil, Uruguay.	Do.
species ?	*Elosia lateristrigata* Baumann.	Leptodactylidae.	Angra dos Reis, State of Rio de Janeiro, Brazil.	Organ Mountains, eastern Brazil.	Brazil.

Species of opalinid	Host species	Family of host	Known geographic occurrence of the opalinid in the host named	Known geographic occurrence of host	Known geographic occurrence of genus of host
Cepedea rubra Carini	*Hyla minuta* Peters.	Hylidae.	Rio de Janeiro, Brazil	Eastern Brazil.	America, including West Indies; one species (several subspecies) in Euro-Asia and North Africa, Papua, Australia.
Do	*Pseudopaludicola ameghini* (Cope).	Leptodactylidae.	Rio de Janeiro, Minas Geraes, Brazil.		
Do	*Paludicola falcipes* Hensel.	Do.	Rio Grande do Sul, Brazil.	Rio Grande do Sul, Brazil.	Continental America from Central America to Patagonia.
Do	*Leptodactylus ocellatus* (Linnaeus).	Do.	Rio de Janeiro, Brazil.	Brazil, Argentina.	Tropical America from Central America to Buenos Aires, West Indies.
borneonensis Metcalf	*Bufo jerboa* Boulenger.	Bufonidae.	Siam, Borneo.	Siam, Borneo.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
celebensis, new	*Bufo celebensis* Schlegel.	Do.	Celebes.	Celebes.	Do.
Do	*Bufo divergens* Peters.	Do.	Borneo.	Natuna Islands, Borneo, Malay Peninsula, Palawan (?), Selangor.	Do.
ciliata, new	*Hyla fuscovaria* Lutz.	Hylidae.	Minas Geraes, Brazil.		America, including West Indies; one species (several subspecies in Euro-Asia and Northern Africa, Papua, Australia.
hasseltii, new	*Leptobrachium hasseltii* Tschudi (=*Megophrys*).	Pelobatidae.	Java.	Upper India and Malay Archipelago.	From the Himalayas to Java and Borneo.
hosei, new	*Nectophryne hosei* Boulenger.	Bufonidae.	Borneo.	Borneo, Malay Peninsula.	Africa, India, Malay Peninsula, Borneo.
lemuriae, new	*Polypedates rhodoscelis* (Boulenger).	Ranidae.	Madagascar.	Madagascar.	Madagascar, Ceylon, southern India, Singapore, Malaysia, Philippines.
longa hispanica Metcalf	*Rana esculenta hispanica* Michaelles.	Do.	Alicante Province, Spain.	Palearctic region.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species in northernmost Australia and another in northernmost South America.

Do	*Rana limnocharis* Wiegmann.	Do.	Darjiling, India.	India, Japan, Formosa, Java.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost Australia and northernmost South America.
longa macronucleata, new.	*Rana vittigera* Wiegmann [=*R. cancrivora*].	Do.	Philippines.	Philippines (?), also Malay Archipelago.	Do.
luzonensis, new	*Rana similis* Günther.	Do.	Rizal, Philippines.	Philippines.	Northern hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost Australia and northernmost South America.
Do	*Rana luzonensis* Boulenger.	Do.	Benquet Province, Philippines.	Highlands of Lepanto, Luzon, Philippines.	Do.
luzonensis aponensis, new.	*Rana magna* Stejneger.	Do.	Mount Apo, Mindanao, Philippines.	Mindanao, Basilan, Mindoro, Luzon, Philippines.	Do.
metcalfi Bhatia and Gulati.	*Bufo melanostictus* Schneider.	Bufonidae.	Punjab, India.	India, southeastern Asia, Malay Archipelago.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
microhylae, new	*Microhyla leucostigma* Boulenger.	Microhylidae.	Borneo.	Perak, Borneo.	India, Borneo.
mogyana (Carini)	*Hyla leucophyllata* (Beiris).	Hylidae.	Angra dos Reis, Brazil.	Nicaragua, Costa Rica, tropical South America.	America, including West Indies; one species (several subspecies) in Euro-Asia and northern Africa; Papua, Australia.
ophis Metcalf	*Rana tigerina* Daudin.	Ranidae.	Punjab, India.	India, Formosa, Billeton Island near Sumatra.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost part of Australia and South America.
philippensis, new	*Bufo quadriporcatus* Boulenger.	Bufonidae.	Sumatra.	Sumatra, Natuna Islands, Borneo, Malay Peninsula up to 240 meters altitude.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
Do	*Bufo philippinicus* Boulenger.	Do.	Caihole River, Ulugan Bay, Palawan, Philippines.	Palawan and Balabec, Philippines.	Do.
plata, new	*Hyla faber* Wied.	Hylidae.	State of Rio de Janeiro, Brazil.	Central and southern Brazil to northern Argentina.	America, including West Indies; one species (several subspecies) in Euro-Asia and northern Africa; Papua, Australia.

Species of opalinid	Host species	Family of host	Known geographic occurrence of the opalinid in the host named	Known geographic occurrence of host	Known geographic occurrence of genus of host
Cepedea pulchra javensis Metcalf.	*Bufo melanostictus* Schneider.	Bufonidae.	Punjab, India.	India, southeastern Asia, Malay Archipelago.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
punjabensis Bhatia and Gulati.	Do.	Do.	Do.	Do.	Do.
saharuna Metcalf.	*Rana esculenta ridibunda* Pallas.	Ranidae.	Turkestan.	Algiers, Turkestan, Beluchistan.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost parts of Australia and South America.
sculpriformis (Ghosh)	*Bufo melanostictus* Schneider.	Bufonidae.	India.	India, southeastern Asia, Malay Archipelago.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
stalkoti Bhatia and Gulati.	*Bufo macrotis* Boulenger.	Do.	Punjab, India.		Do.
siamensis, new	*Bufo asper* Gravenhorst.	Do.	Trong, Lower Siam.	Sumatra, Borneo, Java, Siam, Malay Peninsula up to 1,200 meters altitude.	Do.
spinifera Metcalf	*Oxydozyga lima* (Tschudi).	Ranidae.	Siam.	Eastern India, southern China, Siam, Java.	Southeastern Asia, Java.
virgula (Dobell)	*Polypedates leucomystax* (Gravenhorst).	Do.	Ceylon, southern India.	Ceylon, southeastern Asia.	Madagascar, Ceylon, India, China, Japan, Philippines, East Indies.
species ?	*Aeluroglyphe nasuta* (Günther)	Bufonidae.	Szechwan, China.	Szechwan, China.	Szechwan, China.
species ?	*Eleutherodactylus guentheri* (Steindachner).	Leptodactylidae.	Brazil.	Brazil, Bolivia, Ecuador, Venezuela.	Tropical America.
species	*Rana chalconota* (Schlegel).	Ranidae.	Philippines.	Malay Archipelago.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
Opalina annandali, new	*Rana tigerina* Daudin	Do.	Calcutta, India	India, Formosa, Billiton Island, near Sumatra.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost parts of Australia and of South America.
chattoni Wolf	*Bufo melanostictus* Schneider	Bufonidae.	Saigon, Cochinchina	India, southeastern Asia, Malay Archipelago.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).

caracoidea Bezzenberger.	*Rana cyanophlyctis* Schneider.	Ranidae.	"India."	Tillimanti and Rhamnad, southern India, Ceylon.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost part of Australia and of South America.
caracoidea lahorensis Bhatia and Gulati.	*Bufo melanostictus* Schneider.	Bufonidae.	Lahore, India.	India, southeastern Asia, Malay Archipelago.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
Do	*Rana tigerina* Daudin.	Do.	Do.	India, Formosa, Billeton Island near Sumatra.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost part of Australia and of South America.
japonica Sugiyama	*Cacopus systoma* (Schneider).	Microhylidae.	India.	India.	India.
japonica javensis, new	*Nyctixalus margaritifera* Boulenger.	Ranidae.	Java.	Java.	Java.
larvarum Metcalf	*Rana clamitans* Latreille.	Do.	Nova Scotia, Massachusetts, Philadelphia.	Eastern North America from Florida to Canada, westward into Mississippi Valley and the Great Lakes Basin.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost portion of Australia and of South America.
larvarum Metcalf ?	*Rana catesbeiana* Shaw.	Do.	Do.	North America east of the Rocky Mountains.	Do.
Do	*Rana pipiens* Schreber.	Do.	Ohio, Massachusetts.	Eastern North America, west to the Great Plains and north to Hudson Bay.	Do.
Do	*Rana palustris* LeConte.	Do.	District of Columbia, North Carolina, Michigan, Philadelphia.	North America east of Sierra Nevadas.	Do.
malaysiae, new	*Rana labialis* Boulenger.	Do.	Lower Siam.	Lower Siam, Molucca.	Do.
Do	*Microhyla ornata* Dumeril and Bibron.	Gastrophrynidae.	Rangoon, Burma.	South China, Formosa, Tonkin, Hainan, Burma, Assam, Siam, Annam, Malay Peninsula, India, Ceylon.	Do.
mantellae, new	*Mantella baroni* Boulenger.	Dendrobatidae.	Madagascar.	Madagascar.	Madagascar.
nucleolata, new	*Rana chalconota* (Schlegel).	Ranidae.	Java.	Malay Peninsula, Sumatra, Mentawi Island, Borneo, Java, Celebes, Philippines.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost part of Australia and of South America.

Species of opalinid	Host species	Family of host	Known geographic occurrence of the opalinid in the host named	Known geographic occurrence of host	Known geographic occurrence of genus of host
Opalina nucleolata siamensis, new.	*Rana macrodactyla* (Günther).	Ranidae.	Lower Siam, Tonkin, China.	South China, Tonkin, Burma, Siam, Malay Peninsula.	Nothern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species each in northernmost part of Australia and of South America.
Do	*Rana macrodon* Tschudi.	Do.	Siam, Singapore, Natunas, Engano.	Singapore, Natunas, Engano, Borneo, Java, Sombak, Flores, Batjan.	Do.
obtrigonoidea Metcalf	*Rana palustris* Schreber. Tadpole.	Do.	Massachusetts.	Eastern North America, west to the Great Plains, north to Hudson Bay.	Do.
ranarum (Ehrenberg)	*Rana cyanophlyctis* Schneider.	Do.	Punjab, India.	India.	Do.
ranarum orbiculata, new.	*Rana glandulosa* Boulenger.	Do.	Singapore.	Malay Peninsula, Borneo.	Do.
Do	*Rana temporalis* Günther.	Do.	Ceylon.	Ceylon.	Do.
septentrionalis Metcalf	*Hyla septentrionalis* Boulenger.	Do.	Cuba, Bahamas.	Cuba, Bahamas.	America, including West Indies, one species in Euro-Asia; Australia, Papua.
sudafricana Fantham	*Bufo regularis* Reuss.	Bufonidae.	South Africa.	Africa, south of the Sahara Desert.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
(?) *termitis* de Mello	*Calopiermes* sp. and *Leucotermes indicola*.	Termites.	India.		
triangularis Ghosh	*Bufo melanostictus* Schneider.	Bufonidae.	Punjab, India.	India, southeastern Asia, Malay Archipelago.	Cosmopolitan (except Australasia, Madagascar, the Seychelles).
zeylonica, new	*Polypedates eques* (Günther).	Ranidae.	Ceylon.	Malabar and Ceylon.	Northern Hemisphere, Africa, Madagascar, Seychelles, East Indies, one species in Australia and South America.
species ?	*Rana jerboa* Günther.	Do.	Java.	Burma, Siam, Malay Peninsula, Sumatra, Borneo, Java.	Do.
species	*Rana verrucosa* Gravenhorst.	Do.	Southern India.	Malabar Hills, up to 7,000 feet in the Nilghiris, 4,000 feet in Travancore.	Do.

REVIEW OF THE CLASSIFICATION AND GEOGRAPHIC DISTRIBUTION OF THE OPALINIDAE

The relationships of the Ciliata may be expressed in the following classification, and the phylogeny of the Opalinidae is shown in figure 126.

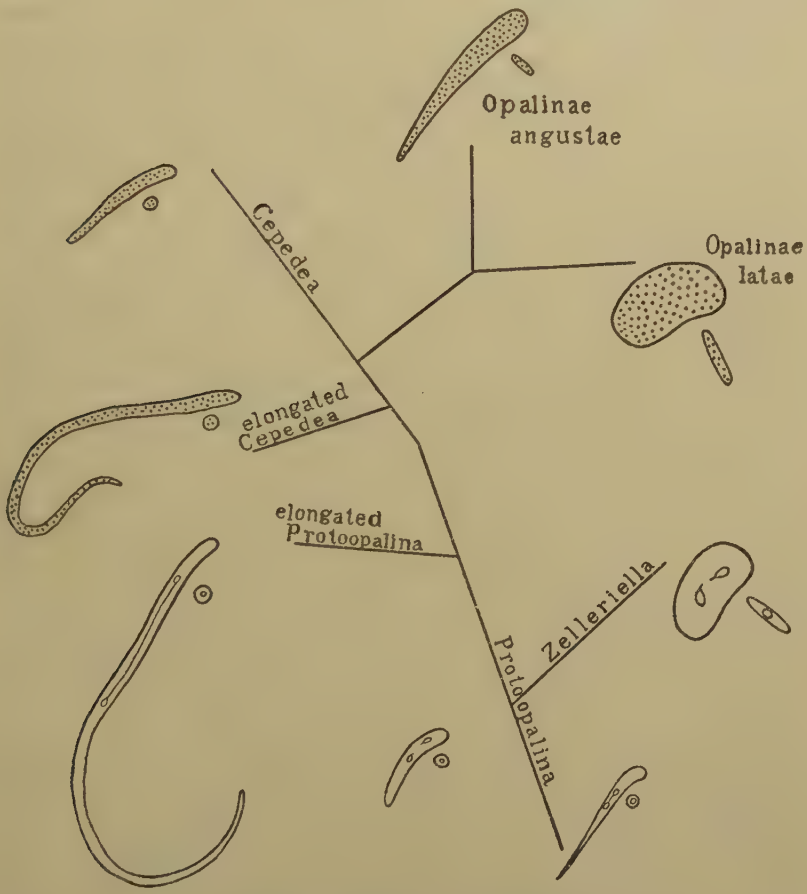

FIGURE 126.—Phylogeny of the Opalinidae

CILIATA
- PROTOCILIATA (Opalinidae)
  - Protoopalininae
    - *Protoopalina*
    - *Zelleriella*
  - Opalininae
    - *Cepedea*
    - *Opalina*
      - *Opalinae latae*
      - *Opalinae angustae*
- EUCILIATA

THE GENUS PROTOOPALINA

In this genus the several species are fairly distinct, more so than in any other genus of Opalinidae. This is in agreement with its archaic character, there having been time for gradual divergence to well-demarcated species. The archaic character of *Protoopalina* is indicated: (1) By its agreement in morphology with the first stages in development of each of the other three genera, *Zelleriella*, *Cepedea*, and *Opalina;* (2) by its wide geographical distribution, Euro-Asia (except southern India and the islands supposed to have been once

FIGURE 127.—Geographic distribution of *Protoopalina*.

parts of the Indian Ocean continent "Lemuria"), Africa, Malaysia, Australasia, South America, Central America and the Antilles, North America (except the northeastern portion) (fig. 127); (3) by the comparative morphology of the four genera, *Protoopalina* being less developed in structure and showing in its series of species how the other genera might readily have been derived from it; (4) by the agreement of the comparative morphological series, *Protoopalina*, *Cepedea*, *Opalina*, with the course of the larval development of the higher genera, complete and convincing evidence of phylogeny (Metcalf, 1923a); (5) by the resemblance of a group of *Protoopalinae* of southern, almost sub-Antarctic distribution (subgeneric group I) to the mother cells of the male gametes in *Protoopalina*, *Cepedea*, and *Opalina*, the only genera the sexual phases of whose development have been studied, and the similar character of the zygotes in *Opalina*. These elongated forms with slender tails, which are naked near the tip, are strikingly alike and seem to be archaic in character. I have regarded the tailed species in *Protoopalina* as constituting the most

archaic subgenus. There is no sharp demarcation. Some species have formae less elongated and others slender and with naked spines (*P. caudata*) and so almost grade into this subgenus. The species that are undoubtedly of this archaic subgenus I are all of southern distribution, Patagonia, Australasia, and southern or tropical Africa. *P. saturnalis*, from Mediterranean fish, shows much resemblance.

Protoopalina, Subgeneric Group I (fig. 128)

P. diplocarya Metcalf, in a leptodactylid, Straits of Magellan.
P. papuensis Metcalf, in a *Hyla*, Dutch Papua.
P. acuta (Raff), in a leptodactylid, Australia.
P. xenopodos Metcalf, in *Xenopus*, Belgian Congo and South Africa.
P. [*capensis*, new species], in a doubtful leptodactylid, Cape Colony, South Africa.
P. appendiculata Fantham, in a *Rana*, South Africa.
P. australis, new species, in a *Hyla*, Australia.

P. africana Metcalf, in a ranid, the Cameroons.
P. nutti Metcalf, in a *Rana*, British East Africa.
P. saturnalis (Leger and Duboscq), in a teleost, Mediterranean Sea.
P. stevensoni Metcalf, in a *Bufo*, Sudan, Africa.

FIGURE 128.—Geographic distribution of *Protoopalina*, subgeneric group I.

The last four species (Metcalf, 1923a, group 2) are less elongated posteriorly than the others, but resemble them. The sub-Antarctic and tropical African distribution of this subgenus indicates that the genus was of southern origin, doubtless in primitive Anura, in the Triassic, Southern Hemisphere, land mass that I have called Equatoria, including Gondwanaland and South America. This archaic subgenus has not spread beyond the limits of its ancestral home.

Protoopalina, Subgeneric Group II, Including Groups 2 and 3 of Metcalf, 1923a (fig. 129)

P. caudata (Zeller), in discoglossids, Europe.
P. c. discoglossi Metcalf, in *Discoglossus*, Europe.
P. c. microhyla Nie, in a gastrophrynid, southwest India.
P. macrocaudata Metcalf, in a discoglossid, eastern Asia.
P. orientalis Metcalf, in a discoglossid, eastern Asia.
P. yunnanensis, new species, in a discoglossid, eastern Asia.
P. caccosterni Fantham, in a gastrophrynid, South Africa.
P. luzonensis, new species, in a gastrophyrnid, Philippine Islands.
P. borneonensis, new species, in a ranid, Borneo.
P. peronii Metcalf, in a leptodactylid, eastern Australia.
P. dorsalis (Raff), in a leptodactylid, western Australia.
P. intestinalis (Stein), in discoglossids, Europe.
P. pelobatidis Metcalf, in *Pelobates*, Europe.
P. hylarum (Raff), in *Hyla*, eastern Australia.
P. bibronii, new species, in an archaic bufonid, southeastern Australia.
P. stejnegeri Metcalf, in a discoglossid, extreme northwestern United States.

FIGURE 129.—Geographic distribution of *Protoopalina*, subgeneric group II.

The typical discoglossid parasites are included here. Through *P. caudata* form *attenuata* and *P. orientalis* the group approaches group I in form. From the archaic species of group I were derived group II, forms many of which have spread to Euro-Asia and are there parasitic in discoglossids, one pelobatid and three gastrophrynids, all but perhaps the last being ancient hosts. The species of this second subgenus that have remained in, or returned to, the sub-Antarctic habitat (Australia) are found in an archaic bufonid, two leptodactylids, and a *Hyla*. I have interpreted the presence of the Protoopalinas of group II in Australia as due to there having been

discoglossids there formerly, *e. g.*, the ancestors of the New Zealand *Liopelma* (Metcalf, 1928*a*). The spread of *Ascaphus* to North America, with its *Protoopalina* of this subgenus, occurred in the Cretaceous period (*cf.* p. 592). The subgenus is as old as the Jurassic period, for it is in Australia, and *Liopelma*, a former host, now barren because of lack of an aquatic, larval stage, is in New Zealand.

Protoopalina, Subgeneric Group III (*Group 5 of Metcalf, 1923a*) (*fig. 130*)

P. montana Metcalf, in a pelobatid, Java.
P. adelaidensis Metcalf, in a *Hyla*, eastern Australia.

FIGURE 130.—Geographic distribution of *Protoopalina*, subgeneric group III.

These are very similar to each other and are but slightly demarcated from group II. Their occurrence, one in Java and one in Australia, agrees with Arldt's conclusion that Java and Australia remained connected through the early Cretaceous after they had separated from Asia and western Malaysia.

Protoopalina, Subgeneric Group IV (*Group 6 of Metcalf, 1923a*) (*fig. 131*)

P. filiformis Metcalf, in a *Rana*, Formosa.
P. tenuis (Raff), in a leptodactylid, eastern Australia.

These elongated forms show some resemblance to the less elongated species *P. africana* and *P. borneonensis*. They apparently evolved at a time before Formosa and Australia definitely parted company, which means probably in the Jurassic period. Tropical Africa and Borneo probably had connection with both Australia and Formosa at that time.

FIGURE 131.—Geographic distribution of *Protoopalina*, subgeneric group IV.

Protoopalina, Subgeneric Group V (Group 7 of Metcalf, 1923a) (fig. 132)

P. regularis Metcalf, in a *Bufo*, tropical Africa.
P. rhinodermatos Metcalf, in a gastrophrynid, South America.
P. longinucleata Metcalf, in a leptodactylid, South America.
P. xamachana, new species, in leptodactylid, Jamaica, West Indies.
P. bufonis Metcalf, in *Bufo*, Cuba, West Indies.
P. mossambicensis Metcalf, in *Rana*, tropical Africa.

FIGURE 132.—Geographic distribution of *Protoopalina*, subgeneric group V.

These tropical species are closely alike. It would seem, then, that this group dates from a time when the American and African tropics were connected (Cretaceous, or more likely Jurassic, period).

Protoopalina, Subgeneric Group VI (Group 8 of Metcalf, 1923a) (fig. 133)

P. scaphiopodos Metcalf, in *Scaphiopus*, a pelobatid, southwestern United States.
P. hammondii Metcalf, in *Scaphiopus*, southwestern United States.
P. mexicana Metcalf, *in Scaphiopus*, northern Mexico.
P. mitotica (Metcalf), in *Ambystoma*, west-central United States.

FIGURE 133.—Geographic distribution of *Protoopalina*, subgeneric group VI.

This compact, sharply distinct, and highly evolved group of species have dumbbell-shaped nuclei. They doubtless evolved from a pelobatid parasite, like *P. pelobatidis*, when the pelobatid host, *Scaphiopus*, had reached North America during the Tertiary period. In the host genus they spread, east of the mountains, as far south as northern Mexico, and one species turning eastward reached the Atlantic coast. *Scaphiopus* is a genus of strange habits, burrowing and seldom seen. Once, in Woods Hole, Mass., where it had been almost unknown, *Scaphiopus holbrookii* appeared, breeding by the many hundreds in surface pools after a good rain. Perhaps this genus may be secretly present in other regions from which it has not been reported.

Protoopalina, Subgeneric Group VII (fig. 134)

P. ovalis Fantham, in *Rana*, South Africa.
P. ovoidea Metcalf, in a gastrophrynid, Texas.

These forms are probably distinct from each other, though they agree in form and dimensions. The nuclei of *P. ovalis* are much more

FIGURE 134.—Geographic distribution of *Protoopalina*, subgeneric group VII.

slenderly oval. The two species seem to form a natural group. *P. xyster* Metcalf, in *Gastrophryne*, from Central America may also belong here.

Protoopalina, Subgeneric Group VIII (Group 9 of Metcalf, 1923a) (fig. 135)

P. formosae Metcalf, in *Bufo*, Formosa.
P. quadrinucleata Metcalf, in *Rana*, Java.
P. axonucleata Metcalf, in *Rana* and *Bufo*, eastern Asia.

FIGURE 135.—Geographic distribution of *Protoopalina*, subgeneric group VIII.

These forms, grouped together to indicate the later steps in the development of *Protoopalina* into *Cepedea*, are of eastern Asian and Malaysian distribution, probably indicating the origin of *Cepedea* there or in some nearby, connected region.

There is one noteworthy feature of the distribution of *Protoopalina*. Only a single species is reported from any of the lands reputed to have been once united to form the Indian Ocean continent Lemuria (Madagascar, the Seychelles, Ceylon, southernmost India). The one instance is *P. caudata microhyla*, in *Microhyla ornata*, from Harnai, Ratnagiri District, south of Bombay, "among mountains." This location is about on the boundary line between the northern Indian and the southern Indian faunas. Its host belongs to a southern family, the Gastrophrynidae. This is the nearest approach to a record of a *Protoopalina* from Lemurian lands. Why are there no more Protoopalinas in these Indian Ocean lands? Study of paleogeographic maps by Arldt and others (fig. 142, *a*) shows Indian Ocean lands connected in Triassic times with Africa and Australia, not with Malaysia. Lemuria is shown connected with Ethiopia, but not with Asia-Malaysia during the Jurassic and early Cretaceous periods. Madagascar separated from Ethiopia after the early Cretaceous and probably did not join it again after this time (see Hewitt, 1922, who suggests temporary late Tertiary connection). The mid-Cretaceous island of India (fig. 143, *b*) is shown uniting with continental Asia late in the Tertiary (fig. 145, *a*) and retaining this connection until the present time. The only suggestion the author can make is that *Protoopalina* was once in Lemuria but was exterminated during the subsidences that broke it into several islands and island groups, but that, before the middle Cretaceous period, somewhat elongated Protoopalinas gave rise to *Cepedea* in Asia-Malaysia, which was in contact with Lemuria for a period long enough to allow Lemuria to become inhabited by *Cepedea* (fig. 143, *a*).

THE GENUS ZELLERIELLA (Fig. 136)

Species distinctions in this genus are difficult, though the genus itself is well demarcated from the other genera. Its origin from *Protoopalina* is indicated by a *Protoopalina* stage in its early development (*cf. Z. brasiliensis* from *Crossodactylus gaudichaudii*, fig. 38). Its geographical distribution, South America, Central America, and Australasia, indicates its origin in the Southern Hemisphere and in that land-complex which in late Cretaceous or early Tertiary times included Australasia, Antarctica, and southern South America, possibly also South Africa early in this period. Comparative numbers in the Australasian and South American regions suggest the origin of *Zelleriella* in the region of greater Antarctica connected with Patagonia and its migration westward from Patagonia to Australia.

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If it arose at the eastern end of this Antarctic land-complex, there remains the question whether the place of its evolution was Patagonia or tropical America. Its hosts are leptodactylids, bufonids, hylas, and dendrobatids, predominantly leptodactylids. The bufonids other than *Bufo* carry *Zelleriella*, so far as known records go, only in South America. They can be left out of account. *Bufo* does not occur in Australia, so it could not have been the host in which *Zelleriella* wandered westward to Australia. *Bufo* was apparently not in Patagonia at the time of this migration. *Bufo* probably arose in southeastern Asia, entered North America, and probably Ecuador also,

FIGURE 136.—Geographic distribution of *Zelleriella*.

by way of the Cretaceous circum-Pacific land-strip (fig. 143, *a*). This land-strip became for the most part fused with North America during the Tertiary period (fig. 144, *b*), but the Isthmus of Panama was not formed until the middle Pliocene, and before that time the land-strip had apparently disintegrated, giving no passage between North and South America. Both *Bufo* and *Hyla*, but not *Zelleriella*, were probably in tropical South America before the leptodactylids, with *Zelleriella*, passed to Australia (see the discussion of the leptodactylids, p. 600).

Zelleriella, the dominant opalinid in leptodactylids, apparently evolved in them in Patagonia, or greater Antarctica of which it was a part, before Patagonia and Brazil united (middle Miocene?) (fig. 145, *a*). It passed to Australia, but not to Asia-Malaysia. This migration occurred, therefore, after the Jurassic, probably after the earliest Cretaceous, period, when Australia and Malaysia were permanently separated. This makes the date of *Zelleriella*'s origin

between the early Cretaceous and the middle Miocene, during the mild climate in Antarctica, which we know from fossils prevailed during the early Miocene and probably earlier. We regard *Zelleriella*, therefore, as a leptodactylid parasite of Antarctic origin in early or middle Tertiary time. When the bar to northward spreading in South America (see discussion of the Leptodactylidae) disappeared, leptodactylids with their Zelleriellas passed northward to tropical America, infected Hylas (sparsely) and Bufos, both already there, with *Zelleriella*, and after the Isthmus of Panama was formed, in the middle Pliocene, they passed on across it in their original leptodactylid, and their new hylid, hosts to Central America and the Antilles which were at that time connected with Yucatan and probably also with Honduras. *Bufo* also may have passed north across the Isthmus at the same time.

Zelleriella is subject to attack from parasites, which are found in the cytoplasm, where they might be mistaken for stages in the nuclear phenomena of the *Zelleriellae* (see fig. 44, *c*; and Metcalf, 1923a, p. 135). I have observed these parasites from Brazil and Texas. Several investigators (Stabler, 1933; Carini and Reichenow, 1935; Chen and Stabler, 1935; Stabler and Chen, 1936; Chen and Stabler, 1936) have made detailed studies on these *Endamoeba* parasites.

THE OPALININAE, COMPRISING THE GENERA CEPEDEA AND OPALINA

We have already seen, while discussing *Protoopalina*, that there is comparative anatomical and developmental evidence to show beyond reasonable question that *Cepedea* developed from *Protoopalina* and that there is indication that this occurred in Asia-Malaysia and before communication of this region with Lemuria had been permanently shut off, for *Cepedea* is in Madagascar, the Seychelles, and Ethiopia, as well as in India and Ceylon. *Bufo* is not in Madagascar and the Seychelles today, so probably never was in Lemuria and could not have been the original host of *Cepedea*. Ranids are today the hosts of *Cepedea* in Madagascar and the Seychelles and probably were its original hosts (*Polypedates?*). They still carry in Asia-Malaysia Protoopalinas of subgeneric group VIII from which *Cepedea* evolved. We place the evolution of *Cepedea*, therefore, in Asia-Malaysia-Lemuria, early in the Cretaceous period, in ranid hosts. Metcalf (1923a) suggested origin in Lemuria, probably in gastrophrynids. *Cepedea* apparently entered South America from Asia and the north before its hosts for this migration had met *Opalina*, for *Opalina* is not in South America; neither is *Rana* in South America, except for one species, *R. palmipes*, which, probably since the middle Pliocene, has sparsely invaded the northernmost parts of the continent. *Opalina* is now abundant in North America and Central America, chiefly in *Rana*, *Bufo*, and hylids, but these have not passed south over the Isthmus with *Opalina*. The most probable explanation seems to be:

(1) That *Bufo*, having adopted *Cepedea* from its ranid hosts, possibly *Polypedates*, in Asia, entered South America from eastern Asia by some route not including North America and Central America, *i. e.*, by the circum-Pacific land-strip, before the close of the Cretaceous period; (2) that in Tertiary times, when this land-strip fused with western North America, *Bufo* entered that continent and later adopted *Opalina*, as it adopts any opalinid when it meets it; (3) that broad *Opalinae* evolved from *Cepedea* in some region not connected with eastern Asia-Malaysia before Bufo's migration to America, *i. e.*,

FIGURE 137.—Geographic distribution of *Cepedea*.

probably in Ethiopia-Lemuria; (4) that *Opalina*, late in the Tertiary, in ranid hosts, entered eastern Asia when the Indian island fused with Asia, and that it passed on, in *Rana*, to Siberia and Alaska and down the Pacific coast of North America, west of the mountains, as far as Central America, but did not go on to South America in spite of a route being open via the Isthmus after the middle Pliocene; (5) that the *Opalinae latae* met hylids for the first time in Central America after the middle Pliocene when they passed northward over the Isthmus of Panama, were then adopted by them, and were changed into the narrow form, *Opalinae angustae*, and were carried by these new hosts northward throughout North America, infecting on the way other Anura. One species, *Hyla arborea*, crossed to Siberia and more southern Asia and with its American, narrow *Opalina* (*obtrigona*) passed on to western Europe and even to northern Africa (see the discussion of the Hylidae). *H. arborea* has evolved several subspecies (usually recognized as species)

CEPEDEA (fig. 137).—The genus *Cepedea* can, perhaps, be divided into several groups, but few of these are sharply demarcated and the divisions are of little interest. As given, they express little more than the author's impressions and these in some cases vague and uncertain.

Subgeneric Group I

In group I Metcalf (1923a) included only Bezzenberger's *C. lanceolata*, in *Rana*, "Asia."

Subgeneric Group II (fig. 138)

Group II is a large group of eastern distribution, except for four species and one subspecies from tropical and subtropical America. I see no reason except their western habitat for separating the latter.

FIGURE 138.—Geographic distribution of *Cepedea*, subgeneric group II.

C. dimidiata (Stein), in *Rana* and *Bufo*, Europe, eastern Asia.
C. d. hawaiiensis Metcalf, in *Rana*, Hawaii, said to have been introduced.
C. d. orientalis Metcalf, in *Rana*, Japan.
C. d. [*paraguayensis*] Metcalf, in *Hyla*, Paraguay.
C. rubra (Carini), in *Hyla* and a leptodactylid, Rio de Janeiro, Brazil.
C. saharana Metcalf, in *Rana*, northern Africa, Turkestan, Beluchistan.
C. buergeri Metcalf, in a ranid, Japan.
C. b. sinensis Metcalf, in *Bufo*, southern China.
C. minor Metcalf, in a discoglossid, France.
C. borneonensis Metcalf, in *Bufo*, Borneo.
C. lemuriae, new species, in a ranid, Madagascar.
C. celebensis, new species, in *Bufo*, Celebes.
C. hasseltii, new species, in a pelobatid, Java.
C. microhylae, new species, in a gastrophrynid, Borneo.
C. siamensis, new species, in *Bufo*, Siam.

C. virgula (Dobell), in a ranid, Malay Peninsula, Ceylon.
C. hosei, new species, in a pelobatid, Java.
C. mogyana (Carini), in *Hyla*, Rio de Janeiro, Brazil.
C. occidentalis Metcalf, in *Rana*, tropical Central America.
C. floridensis Metcalf, in a pelobatid, Florida.
C. obovoidea Metcalf, in *Bufo*, Florida.

Subgeneric Group III

C. spinifera Metcalf, in a ranid, Java. Its well-defined posterior spine, shown in many but not all individuals, is noteworthy. A few Protoopalinas, *e. g.*, *P. stevensoni*, show the same thing.

Subgeneric Group IV

Group IV includes two species that resemble each other more than they do any other species:

C. globosa Metcalf, in a hylid, Central America.
C. baudinii Metcalf, in *Hyla*, Central America.

Subgeneric Group V

This group includes a single eastern species with two subspecies:

C. pulchra Metcalf, in a gastrophrynid, Cochinchina.
C. p. japonica Metcalf, in *Rana*, Japan.
C. p. javensis Metcalf, in *Bufo*, Java.

Subgeneric Group VI, A

These are species of more or less elongated form and irregular shape, the latter due, perhaps, to an unusually delicate pellicle. Many of them have their dividing nuclei of unusually slender and elongated shape.

C. phrynomantidis Metcalf, in a gastrophrynid, East Africa.
C. madagascariensis Metcalf, in a ranid, Madagascar.
C. madagascariensis [of *Hyperolius*] Metcalf, in a ranid, West Africa.
C. magna Metcalf, in Bufo, West Africa.

Subgeneric Group VI, B (fig. 139)

C. formosae Metcalf, in *Bufo*, China, Formosa.
C. philippensis, new species, in *Bufo*, Philippine Islands.
C. mexicana Metcalf, in *Rana*, Mexico.
C. luzonensis, new species, in *Rana*, Philippine Islands.
C. l. aponensis, new subspecies, in *Rana*, Philippine Islands.
C. ciliata, new species, in *Hyla*, southern Brazil.
C. cantabrigensis Metcalf, in *Rana*, northwestern North America.
C. multiformis Metcalf, in *Hyla*, Central America.
C. multiformis [of *Polypedates schlegelii*] Metcalf, in a ranid, Japan.
C. seychellensis Metcalf, in a ranid, Seychelles Islands.
C. dolichosoma Metcalf, in *Bufo*, Central America.
C. sp. ?, in *Hyla*, Texas.
C. longa (Bezzenberger), in *Rana*, "Asia."
C. l. hispanica Metcalf, in *Rana*, Spain, northern India.
C. ophis Metcalf, in *Rana*, Formosa, East Indies.
C. segmentata Metcalf, in a ranid, Cochinchina, East Indies.

FIGURE 139.—Geographic distribution of *Cepedea*, subgeneric group VI, B.

Elongated Cepedeas occur, on the one hand, in Africa and Madagascar, and on the other hand chiefly in lands once a part of or accessible from the Cretaceous circum-Pacific land-strip. The significance, if any, of this fact we do not discuss, for all grouping within the genus is too faintly indicated to be worth much emphasis.

Subgeneric Group VII

C. plata, new species, in *Hyla*, Rio de Janeiro, Brazil.

This species is so flat that it might be taken for an *Opalina*, but its narrowness and the impression from the general shapes in the infections indicate that it is *Cepedea*.

Species Not Assigned to Any Group

C. flava (Stokes) is omitted because of wholly inadequate description. Four species, described by others, I have not seen and so hesitate to place them in any of these groups:

C. scalpriformis (Ghosh), in *Bufo*, India.
C. sialkoti Bhatia and Gulati, in *Bufo*, Punjab, India.
C. metcalfi Bhatia and Gulati, in *Bufo*, Punjab, India.
C. punjabensis Bhatia and Gulati, in *Bufo*, Punjab, India.

OPALINA (figs. 140 and 141).—The genus *Opalina* we have divided into (1) broad species, found in the Eastern Hemisphere, with some emigrant species that have penetrated to North America and are now living on the Pacific coast, west of the mountains and in the tropical lands to the south, often called Central America, and (2) narrow species which I have suggested were developed in the Hylas after they

arrived in the north, coming from South America in the middle Pliocene. These *Opalinae angustae* have been adopted by other North American hosts and are spread throughout the continent. This subgenus of narrow species, recently evolved, has developed few sharply demarcated species and its taxonomy is difficult, like that of the also comparatively modern genus *Zelleriella*. As to the *Opalinae latae*, I feel that one can recognize more or less vaguely some subgrouping. There seem to be at least *ranarum*-like forms and *japonica*-like forms,

FIGURE 140.—Geographic distribution of the *Opalinae latae*.

the latter distinguished by curved and often abruptly pointed posterior ends and by smaller nuclei.

In the group of species resembling *O. ranarum* we may place the following:

O. ranarum (Ehrenberg), in *Rana* and secondary hosts, Europe.
O. r. smithi Metcalf, in *Bufo*, Japan.
O. r. orbiculata, new subspecies, in *Rana*, Ceylon, Singapore.
O. cincta Collin, in *Bufo*, Europe.
O. bufoxena Metcalf, in Bufo, Manchuria.

In the *japonica* group may be reckoned the following:

O. japonica Sugiyama, in *Rana*, Japan.
O. japonica (?) Metcalf, in *Rana*, Java.
O. coracoidea Bezzenberger, in *Rana*, "Asia," Ceylon.
O. c. lahorensis Bhatia and Gulati, in *Bufo*, India.
O. camerunensis Metcalf, in a ranid, Cameroons, Africa, and two species from the Pacific coast of North America.
O. draytonii Metcalf, in *Rana*, California.
O. panamensis Metcalf, in *Bufo*, Panama.

O. natalensis Metcalf, in a ranid from the Sudan, is almost intermediate between the *ranarum*-like species and the *japonica*-like species. The nuclei are large, but the shapes of some individuals are *japonica*-like. Other individuals are unique in form.

O. rotunda Metcalf, in *Rana*, from Siamese Cambodia, has numerous individuals that more or less resemble *O. japonica*, but its nuclei are a little larger.

O. annandali, new species, in *Rana*, from Calcutta, India, has small nuclei. Its posterior end is sometimes abruptly pointed. Its shape

FIGURE 141.—Geographic distribution of the *Opalinae anguetae*.

is frequently irregular, as is true also of *O. natalensis* and *O. rotunda*. These three might be regarded as related and as somewhat intermediate between the *japonica* group and the *ranarum* group. *O. zeylonica*, new species, in a ranid from Ceylon, with small nuclei and irregular shape, may also belong here.

O. mantellae, new species, in *Mantella* (one of the Ranidae) from Madagascar, has small nuclei, is *ranarum*-like in shape except for a few small, narrow individuals. Its possible relationships are not suggested.

O. chattoni Weill, in *Bufo*, from Cochinchina, has, in the cysts and young forms only, peculiar nuclei with each a single large nucleolar mass and a large, clear, chromatin skein. It seems a very distinct species. *O. nucleolata*, new species, in *Rana*, from Java, has usually a single nucleolar mass in its nucleus, though sometimes two are found. No such chromatin skein as in *O. chattoni* is found. These species are probably related. Their nuclei are large and their shape is like that of *O. ranarum*. Two species of *Rana* from

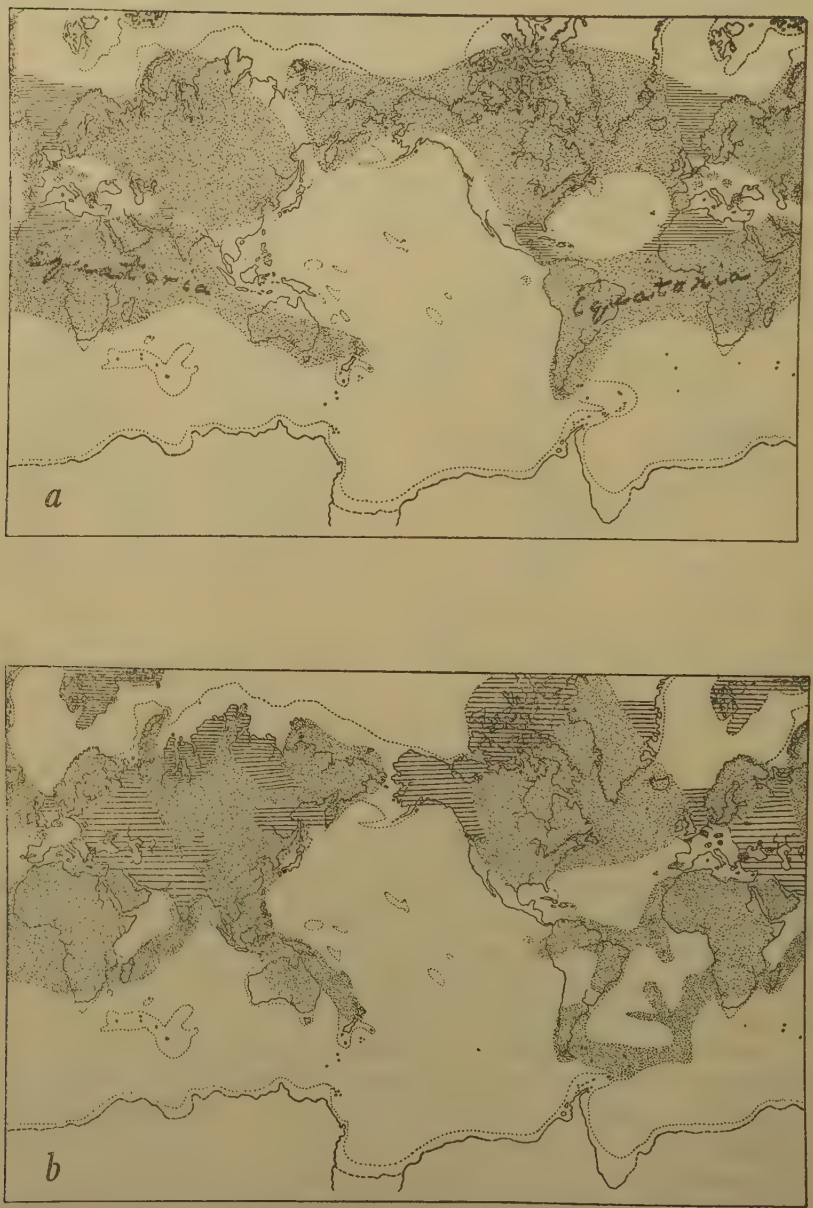

FIGURE 142.—*a*, Triassic continents (from Arldt): The stippled areas are late Triassic, the line shading indicating additional, earlier Triassic land; *b*, Jurassic continents: The stippled areas are late Jurassic, the line shading indicating other, early Jurassic lands.

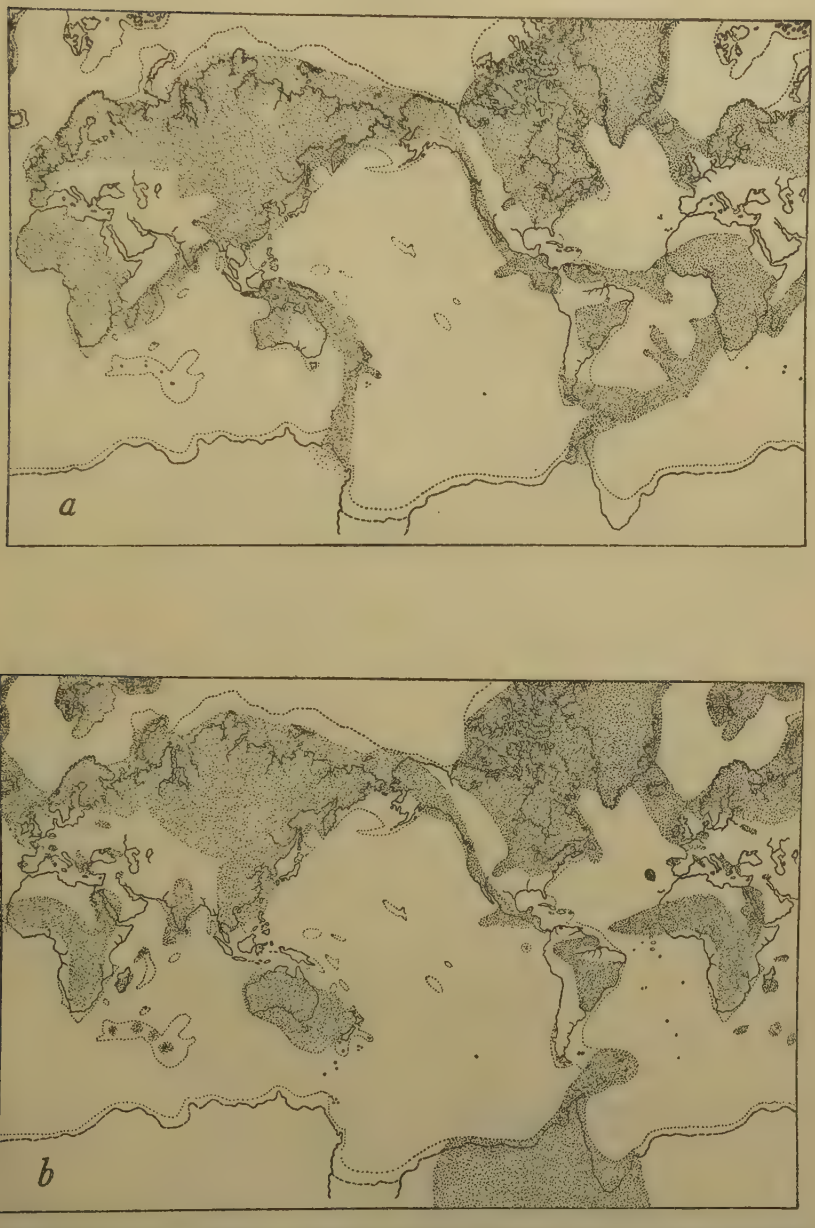

FIGURE 143.—*a*, Early Cretaceous continents; *b*, Middle Cretaceous continents. (After Schuchert.)

FIGURE 144.—*a*, Late Cretaceous lands (the southern Pacific may have been, instead or also, Eocene); *b*, Early Tertiary lands (after Arldt, modified). The trans-Pacific lands shown in *a* probably were present in the Eocene.

FIGURE 145.—*a*, Late Tertiary continents; *b*, Pleistocene continents (after Arldt).

southeastern Asia (Tonkin, China, and Lower Siam) bear Opalinas of similar shape to *O. nucleolata*, and their nuclei, much smaller, bear each a single nucleolar mass. They may be classed as *O. nucleolata siamensis*, new subspecies. *O. malaysiae*, new species, in a Siamese *Rana*, is in form intermediate between the *japonica* group and *O. nucleolata*. Its nuclei are small and show numerous small nucleolar disks, as is usual, instead of one or two overemphasized ones.

I make no attempt to suggest the affinities of the huge *O. lata*, described by Bezzenberger. Observation of many individuals in whole infections is necessary for this.

FIGURE 146.—Mercator's projection map showing land areas in dashed shading outlined by continuous line, except that unexplored shores of Antarctica have dashed lines; ocean shallows as stippled areas outlined by dashed lines on the deep-seaward side; deeper ocean areas unshaded and outlined by dashed lines.

THE CLASSIFICATION AND DISTRIBUTION OF THE ANURA

PIPIDAE:

Pipinae, the Guianas, no opalinids found.

Xenopodinae, tropical and southern Africa. *Protoopalinae* of subgenus I.

DISCOGLOSSIDAE:

Discoglossinae, Euro-Asia with northern Africa. *Protoopalinae* of subgenus II.

Ascaphinae, extreme northwestern United States. *Protoopalina* of subgenus II.

Liopelminae, New Zealand. No opalinids, because no larvae.

PELOBATIDAE: Western Europe, southern and southwestern Asia, Malaysia, Papuasia. *Protoopalinae* of subgenera II, III, and VI, and *Opalinae angustae* (lately evolved in North America).

ARCHAIC BUFONIDAE: Australia, Neotropics, Ethiopia, India, Java. Opalinids not studied, except one *Protoopalina* and two Zelleriellas, in South America.

Bufo, cosmopolitan, except Madagascar and Australasia including Papua and New Zealand. Host to all opalinids it meets.

HYLIDAE: America, Australasia, except New Zealand, one species (with several subspecies) in Europe and eastern Asia. *Protoopalina, Zelleriella, Cepedea, Opalinae angustae.*

LEPTODACTYLIDAE; Tropical America, Australasia (except New Zealand), Africa (?). *Protoopalina, Cepedea,* and especially *Zelleriella.*

GASTROPHRYNIDAE: Neotropics, Ethiopia, Madagascar, southern India, southeastern Asia, Amboina, Papua. *Protoopalina, Zelleriella, Opalina,* and especially *Cepedea.*

RANIDAE:

Ceratobatrachinae, Solomon Islands. Opalinids unknown.

Raninae, Eastern Hemisphere (except Australia, Tasmania, and New Zealand), North and Central America, one species each in the northernmost portions of Australia and South America, respectively. *Protoopalina, Zelleriella* (4 cases), and especially the multinucleate genera *Cepedea* and *Opalina.*

Dendrobatinae, Neotropics. *Zelleriella.*

Mantellinae, Madagascar. *Opalinae latae.*

Cardioglossa, classification doubtful, western Africa. Opalinids not studied,

The Opalinidae arose as *Protoopalinae* of subgenus I in archaic Anura, perhaps Pipidae, apparently in the Southern Hemisphere, and spread to all portions of the earth capable of supporting Anura, except possibly Lemuria where their present absence is unexplained. As the Anura evolved into their several families, their commensal opalinids evolved to their present diversity. The interrelationships and the course of the evolution of the families, subfamilies, genera, and species of the Anura are by no means understood, though some things are indicated. (Fig. 147.)

THE PIPIDAE (Fig. 148)

The Pipidae seem the most archaic of extant Anura, the African and South American genera having diverged to different subfamilies. The Papinae (Guianas) have not been found carrying opalinids, probably because of the absence of free-living, browsing, vegetarian, aquatic larvae, for it is in this stage of its life history that an anuran becomes infected with encysted opalinids from the recta of the hosts. The Xenopodinae (Africa) bear Protoopalinas belonging to the most ancient subgeneric group, I. It seems natural that the most archaic family of Anura should bear the most archaic opalinids, members of the most archaic subgenus of the most ancient genus.

THE DISCOGLOSSIDAE, OR BELL TOADS

The Discoglossidae, which, according to Stejneger, probably arose in southeastern Asia, near the eastern end of the Himalayan highlands, spread in three directions: (1) The Discoglossinae spread northward, inhabiting western Europe and northern Africa (*Bombina, Discoglossus, Alytes*) and also eastern Asia (*Bombina*). This was one migration, by routes north of the Himalayas, the eastern and western genera becoming separated by the development of desert conditions

between. The opalinids of the subfamily Discoglossinae are Protoopalinas of subgenus II. (2) The Ascaphinae spread northeastward and across to extreme northwestern North America by way of the land-strip that in Cretaceous times stretched north from eastern Asia

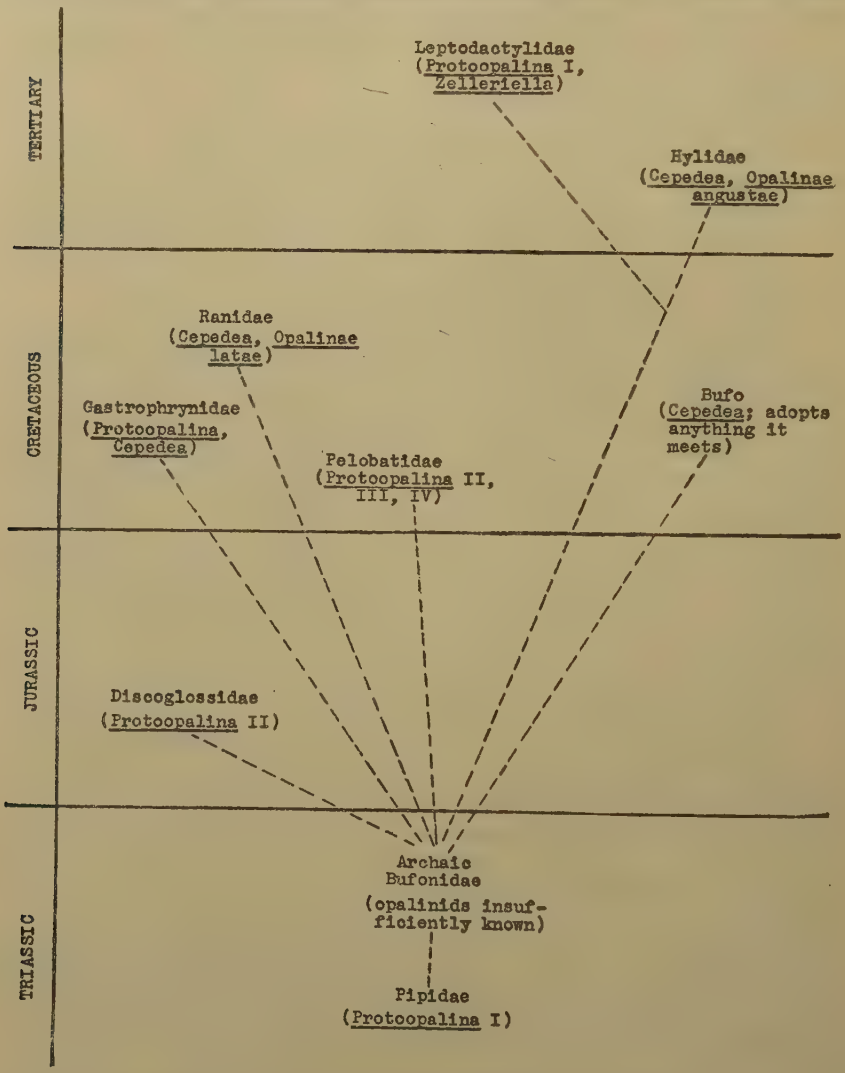

FIGURE 147.—Chart suggesting possible phylogeny of the Anura.

and east and then south, bounding the northern Pacific Ocean (fig. 143, *a*). Although this land-strip in Tertiary times united with North America (fig. 144, *b*) its discoglossids never passed farther east onto the main part of the North American continent even when the way opened, but they remained on the narrow land-strip, being already decadent, having retired to the mountains and living in and near the

cold, glacial streams. Their tadpoles carry Protoopalinas of subgenus II brought from Asia, but in the cold water of these streams their metamorphosis is delayed until the second year, so that opalinid

FIGURE 148.—Geographic distribution of the Pipidae.

FIGURE 149.—Geographic distribution of the Discoglossidae.

infection is from the tadpoles of the former year to the young tadpoles of the later year. As is so often the case when tadpoles live into the second season, the adult anurans are not infected (cf. *Rana catesbeiana*, *R. clamitans*). The evolution of *Ascaphus* into its decadent character

we thus consider to have started as early as the Cretaceous period and to have been completed before, in the early Tertiary union of the Pacific land-strip with the North American Continent occurred. (3) The Liopelminae spread southeastward across Malaysia, through Australia, to New Zealand. This wandering occurred before Australia and New Zealand separated from Malaysia and Asia, in other words, apparently in Jurassic times (fig. 142, *b*). *Liopelma*, another decadent genus, has completely lost its aquatic larvae and so has no opalinids.

FIGURE 150.—Geographic distribution of the Pelobatidae.

But its ancestors, in passing across Australia, left their Protoopalinas of subgenus II, and these are found there today resident in *Hyla*. There is a further point of considerable interest. *Hyla* came to Australia from South America and at a time later than the separation of Australia from Malaysia, for *Hyla* is unknown from Malaysia. It seems likely that the ancestors of *Liopelma* persisted in Australia long enough to meet and infect *Hyla*, though the infection might have been from *Liopelma* through a primitive bufonid or a leptodactylid (?) to *Hyla*.[6] The point of interest is that discoglossid Protoopalinas are still in Australia, though the discoglossids themselves are gone.

Discoglossids have come into contact with *Cepedea* but have not adopted it. Similarly they have been in contact with *Opalina*, but only in two or three instances have individuals infected with *Opalina* been reported (from Europe), probably temporary infections. Discoglossids have not been in contact with *Zelleriella*.

[6] It is not likely that the Hylidae and the Leptodactylidae entered Australia together, coming from Antarctica. The Hylidae may have taken a more northern trans-Pacific route from tropical South America. In this case the migration was probably earlier (late Cretaceous or, say, Eocene; fig. 144, *a*) than that of the leptodactylids (Tertiary; fig. 144, *b*). See p. 598.

THE PELOBATIDAE (Fig. 150)

These toads are found today in the lands north and east of the Mediterranean Sea, in Asia south and southwest of the Himalayas, in Malaysia and Papua, and in North America. Their origin and spread seems to parallel that of the Discoglossidae. They probably evolved in India or in southeastern Asia in Cretaceous times after Australia had separated from Asia. They seem to have passed to the Mediterranean lands by a route that lay either to the north or to the south of the Himalayas; to have passed to Malaysia and Papua during the

FIGURE 151.—Geographic distribution of the Bufonidae, other than *Bufo*.

time of fluctuations when the Malaysian and Papuan connections were repeatedly formed and broken (late Tertiary or Quaternary, fig. 145); to have passed during the earlier or later Tertiary by way of Siberia and Alaska to North America, this northeastward migration including only the one genus, *Scaphiopus*, or rather its ancestors. The opalinids of the Pelobatidae were originally Protoopalinas of subgenera II and III and they still are, except for the very compact group of species, subgenus VI, evolved in *Scaphiopus* since its migration to North America, and except also for certain adventitious, late Tertiary infections in *Scaphiopus* in North America by two species of *Zelleriella* and two species of narrow *Opalinae*. These infections were apparently since the middle Pliocene, *i. e.*, since the Isthmus of Panama was formed.

THE ARCHAIC BUFONIDAE (Fig. 151)

These genera I am discussing apart from the remarkable genus *Bufo*. Their present distribution indicates an origin in the Southern Hemisphere in pre-Cretaceous times, before Australia separated from

Malaysia. But these forms have not been sufficiently studied and their opalinids are unknown, except for one Australian species which carries *Protoopalina*, and except also for some few of the species from the highlands of western South America which carry the modern genus *Zelleriella*, indicating late infection through contact with leptodactylids. Full knowledge of their parasites might solve some of the puzzles that we now leave without discussion.

Bufo will be treated later.

THE HYLIDAE (Fig. 152)

The Hylidae apparently evolved in tropical America in the heavy forest area for which they are so well adapted. Their ancestors were probably archaic Bufonidae, since pelobatids are not today present in tropical America and apparently never were. What their opalinids were is not clear from any available evidence. The Australian Hylas today bear *Protoopalina* of subgenus II. The South American forms carry *Cepedea* and *Zelleriella*, the latter, I believe, a late introduction (see discussion of the Leptodactylidae). Their Cepedeas probably were introduced to South America by *Bufo* during the Cretaceous (cf. discussion of *Bufo*). No Hylas in South America bear *Opalina*. Apparently Hylas met *Opalinae* for the first time in the middle Pliocene period after the Isthmus of Panama had been formed and they had crossed into North America. Meeting there Bufos and Ranas, immigrant from Asia, with their Asiatic broad Opalinas, they adopted these and changed them into *Opalinae angustae*. Spreading over North America these Hylas in turn gave of their narrow *Opalinae* to certain Ranas and Bufos, to a pelobatid and to a gastrophrynid. The Isthmus of Panama was completed, apparently in the middle Pliocene (Vaughan, 1919), so that the colonization of North America by hylids and their subse quent wandering across Alaska, Siberia, and on to western Europe was accomplished within the late Pliocene and the Pleistocene periods, an extensive spread in what is geologically a rather brief time. Only a single species, *Hyla arborea*, with its half dozen or so subspecies, entered Euro-Asia bearing *Opalina obtrigona*, an American narrow *Opalina*. *Hyla arborea* is very closely related to northern North American Hylas. It is not closely similar to any species in Australia.

Hyla is a vigorous, dominant form to which such an extensive and comparatively rapid spread might be possible. Its behavior is in marked contrast to that of the decadent *Ascaphus* and the semidecadent *Scaphiopus*, both of which were in North America earlier than *Hyla* and merely managed to persist by hiding, one of them (*Ascaphus*) not having spread at all since the Cretaceous period, and the other (*Scaphiopus*) spreading east of the mountains only through Western United States and northern Mexico, except

for one species, *solitarius*, which spread to the Atlantic coast. Examples of other vigorous, dominant Anura are *Bufo*, *Rana*, and the family Leptodactylidae. *Rana* is a vigorous genus of a vigorous family. *Bufo* is a vigorous genus in an otherwise seemingly decadent family.

The place of origin of the Hylidae deserves a little further comment. The comparative numbers of species in the different regions, two score in Australasia, rather more in North America, and very many in South America seem to indicate origin in South America. On the other hand, the diversity of species north of the Isthmus, forming a number of "genera," would seem to indicate a long period of evolution and would point to a longer residence in the north than

FIGURE 152.—Geographic distribution of the Hylidae. The stippled areas indicate the presence or former presence of one species, *H. arborea*, and closely related forms usually classed as a separate species, but their classification as subspecies of *H. arborea* would better express the true relationships. The dot in eastern Africa indicates the reputed but very doubtful occurrence in Abyssinia of *H. wachei*, not closely related to *H. arborea*.

in the South American forests. Two considerations, however, should be held in mind. North of the Isthmus hylids have been exposed to more varied environmental conditions than in the South American forests, especially during the climatic fluctuations of the successive periods of glaciation, and, in the second place, the distinctions between the genera of Hylidae are not worthy of much emphasis from the standpoint of evolution.

The complete absence of hylids in southern South America, especially when we remember that cold has not prevented their penetrating as far north as Great Slave Lake, is noteworthy and significant. It is in accord with the fact that in a good many other groups the

central South American fauna is very distinct from that of southern South America, the dividing line being from about the mouth of the Rio de la Plata to north-central Chile. There is abundant faunal and floral evidence of a former bar to the spreading of species of animals and plants northward or southward across this line. It seems most likely that this obstacle to migration was salt water, an arm of the sea or an ocean channel, but satisfactory geologic evidence of this has not been presented. The bar, of whatever sort, was effective in many groups of animals and plants. South of this obstruction the fauna and flora show resemblance to those of Australia and New Zealand in very many items, more than they do to the animals and plants of central South America. Von Ihering made this point clearly, and it has since been confirmed by many others, *e. g.*, Eigenmann in his studies of teleosts.

By what route did *Hyla* pass between tropical America and Australia? Not by any Northern Hemisphere route, for hylids are not now in Euro-Asia except one species, with its several subspecies, a comparatively recent immigrant from North America; not by way of Patagonia and Antarctica, for no hylids are today in Patagonia. This restricts them to a trans-Pacific route across the southern Pacific Ocean, by way of the extensive lands present there during the Cretaceous period (Haug, 1907–1911; Scharff, 1911; Arldt, 1907; Berry, 1930; Joleaud, 1931; and many others). Study of the Pacific islands and the ocean bottom shows many branching ridges (fig. 146), interconnecting in many ways. Former rising and sinking of Pacific lands is indicated by several things. The coral islands indicate much depression; the Hawaiian ridge is today rising at one end and sinking at the other; volcanic action and earthquakes, frequently associated with changes of elevation, are and have been present through the Pacific area; it is generally recognized that repeated changes of elevation have occurred among the Malay islands, especially along their southwest-northeast axis (Merrill, 1931). The hypothesis of connection between the South American tropics and Australasia, probably northern Australasia, by means of land ridges, or perhaps land waves such as are illustrated today in Hawaii, is not a far-fetched one. But, if this was the migration route for the Hylidae from America to Australasia, why are there not at least some relic forms among the central and eastern Pacific islands? We find Hylas in the islands only of the Papuasian region of the extreme western Pacific. Nothing but subsidence of the southern Pacific lands seems adequate to account for such extermination of former Anura. The repeated formation, expansion, and shrinking of the Arctic (and Antarctic?) ice sheets are estimated to have caused fluctuations of ocean level of over 60 feet, but that could be, of course, only a minor factor for islands with mountains of considerable elevation. Evidence of a tropical

trans-Pacific land route between South America and Malaysia or Australasia is furnished by corals, Crustacea, spiders, Mollusca, and Foraminifera, chiefly by fossils of these groups; and the paleontological evidence places the communication in the Eocene (see Berry, 1930). Joleaud (1931) routes the trans-Pacific land bridge via Papua, Bismarck Archipelago, Marshall Islands, New Hebrides, Fiji, and Cook Islands. With frequent changes in extent of its connections this bridge may have existed from the late Cretaceous through, or perhaps beyond, the Eocene.

THE LEPTODACTYLIDAE (Fig. 153)

Tropical America, both south and north of the Isthmus of Panama, abounds in leptodactylids. They are well represented, a score or more species, in Tasmania, Australia, and Papuasia, and are found nowhere else except for a couple of forms that have spread into southern Texas and perhaps one genus, two species, in South Africa. This form, *Heleophryne*, has primitive parasites, Protoopalinas of subgenus I, and these do not help solve the puzzle of the presence in South Africa of a single genus, a reputed leptodactylid, so far from the proper home of the Leptodactylidae. Stejneger is inclined to consider *Heleophryne* a ranid with arrested development. If it is a true leptodactylid its distribution is a serious puzzle.

Judged from the great number of species and genera in tropical America, that would seem the ancestral home of the family, but there are reasons for questioning this. *Hyla* is not in Patagonia. If *Hyla* and leptodactylids were together in Brazil, before the trans-Argentine sea (?) was present, why did they not pass together to Papua and Australia? Leptodactylids are in Patagonia today, and at some time since Australasia separated from Asia-Malaysia they entered Australasia. They are today an active, vigorous dominant family, taking, in Australia and South America, a place similar to that of the Ranas in other lands. *Rana* is not in these two continents, except for a single species in the extreme north in each continent. Leptodactylids were apparently the characteristic Anura of Antarctica and connected lands (Australia, Patagonia) during the early Miocene or earlier, when the Antarctic climate was mild and moist, as indicated by its fossil flora.

We may reconstruct the history of the Leptodactylidae about as follows: They arose from ancestors common with the Hylidae. The Hylidae evolved to the north of the trans-South American sea (or whatever it was), which effectively separated tropical South America from Patagonia, and became adapted to tropical rain-forest conditions. The Leptodactylidae evolved in Patagonia, or in lands farther south and west (Antarctica). Later, when they spread over all tropical and south-temperate South America, they gave rise to numerous

genera of diverse character fitting their environment, more diverse than in the rain forest. During the time when Patagonia, Antarctica, and Australasia were connected, *Zelleriella* developed from *Protoopalina* originally present, this evolution being probably rapid, so that the leptodactylids carried *Zelleriella* wherever they spread, except for *Heleophryne* in South Africa, perhaps not a leptodactylid. When Patagonia became connected with tropical America, the leptodactylids passed northward with their Zelleriellas. At about the middle Pliocene the Isthmus of Panama was formed and leptodactylids and *Zelleriella* passed on to Central America and to the Antilles, which were then connected with Yucatan or Nicaragua or both. When

FIGURE 153.—Geographic distribution of the Leptodactylidae.

the leptodactylids reached Brazil they met Hylas and Bufos and gave them *Zelleriella*, *Hyla* receiving these sparingly, *Bufo* with more generous hospitality. The Australasian lands, after they were stocked by leptodactylids and *Zelleriella*, had no northern connections, and even New Zealand was no longer connected, so that leptodactylids and *Zelleriella* did not reach New Zealand, Malaysia, and Asia.

Leptodactylids have never been in Euro-Asia, for if they ever had been and had become exterminated in some strange way (a wholly improbable hypothesis) they would have given their Zelleriellas to at least the Asian Bufos, a genus always hospitable to any genus of opalinid. The leptodactylids and *Zelleriella* are of Antarctic origin, using the word Antarctic to cover, not only the present, restricted Antarctica, but the wider continent with its connected lands including Australasia and Patagonia. Of direct connection between Antarctica and South Africa the Opalinidae give us only little, if any, evidence.

Connection between South America and Africa, of which there is general faunal evidence, may have been in the equatorial or sub-Antarctic zone, rather than by way of Antarctica itself.

Other opalinids reported from leptodactylids are Protoopalinas, their original guests, and Cepedeas, both infections occurring in South America. The latter infection, by *Cepedea rubra* in three individuals of *Paludicola falcipes*, from Minas Geraes, Brazil, being the only report of *Cepedea* from a reputed leptodactylid, seems to cast doubt upon the status of *Paludicola* as a leptodactylid and to suggest its relegation to the Bufonidae where it was long classed, but I know no competent herpetologist who receives this suggestion kindly. (There was no error in identification of these specimens; they are now in the United States National Museum.) The leptodactylids never met *Opalina* until after the middle Pliocene in Central America, and they have not yet adopted it. *Cepedea* entered South America probably with *Bufo* (see discussion of *Bufo* later), but leptodactylids, though in contact with it, have not welcomed it. They are resistant to infection by any of the multinucleate opalinids.

THE GASTROPHRYNIDAE (Fig. 154)

The distribution of the Gastrophrynidae is puzzling. They are not a numerous family and seem to be rather feeble amphibians. They may at one time have had a wide distribution. Their presence in Africa and Madagascar indicates origin before the separation of these two lands, *i. e.*, before the middle Cretaceous.[7] Their presence in Ceylon and India agrees with this date of origin. Their absence from Australia is evidence (not conclusive, of course) that they were not in Asia-Malaysia much earlier than this, for Australia probably had Malaysian land connection earlier than the Cretaceous (in the Jurassic). But it is unsafe to rely upon negative evidence from such an apparently more or less decadent family. Australian climatic conditions have suffered great changes since the early Cretaceous and probably there was much subsidence and elevation, and forms without much vigor and adaptability might well have been exterminated. The presence of several genera of two subfamilies in the Papuan region would agree with a hypothesis of long residence there and perhaps of former residence in Australia, when Papua and Australia were connected. The fact that gastrophrynids are now in southern Siam, Borneo, the Philippines, and Papua would indicate former spread across the Malayan islands with later extermination in most of these islands during the frequent late Tertiary fluctuations in this region, especially during the Pleistocene. The presence of several genera of gastrophrynids in tropical America might be explained by the commonly postulated Afro-American land connection usually thought to

[7] See Hewitt, 1922, who suggests, for reasons not stated, that Ethiopia and Madagascar were again connected for a brief period in the late Tertiary.

have been not later than the early Cretaceous, if not in the Jurassic period. Migration from eastern Asia by way of the Cretaceous circum-Pacific land-strip, which Arldt (1907), Scharff (1911), and others think extended well to the south on both the Asian and American ends, is equally a possibility, but there are no eastern Asian or western North American species today to lend support to this hypothesis.

The opalinids of the gastrophrynids are: *Protoopalina* (North and South America, Africa, India, Philippine Islands), *Zelleriella* (Central

FIGURE 154.—Geographic distribution of the Gastrophrynidae.

America, southern South America), *Cepedea* (Cochinchina), *Opalinae latae* (India), *Opalinae angustae* (southeastern United States, adopted after the middle Pliocene from Hylas directly or indirectly). I do not see that they give evidence as to the place and time of origin of their hosts.

THE RANIDAE (Fig. 155)

This is a large family of many genera, found abundantly in all lands with suitable climate, except Australia, Tasmania, New Zealand, and South America. Australia has one *Rana* in the extreme north, and South America has one *Rana* and two other genera of Raninae represented in the northernmost regions. The family is a dominant one and *Rana*, wherever the family is well represented, is its chief genus.

The opalinid parasites of the family are: *Protoopalina* (tropical and southern Africa and Malay islands), *Zelleriella* (rare), *Cepedea*, and *Opalina*. *Zelleriella* is a guest recently adopted by one Californian *Rana*. It is also borne by *Prostherapis* and *Phyllobates* on the northern-

most edge of South America. *Cepedea* and especially *Opalina* are the characteristic parasites.

The family, other than *Rana*, is tropical and east Asian in distribution and probably arose in some palaeotropical region before Africa and Madagascar separated, that is, before the mid-Cretaceous. The distribution of these ranids other than *Rana* is somewhat similar to that of the bufonids other than *Bufo*, except that the archaic bufonids are lacking in Madagascar and Papua and present in Australia, while the older (?) ranids are absent only from Australia. *Rana* seems to have been comparatively recently evolved, as is indicated by its representation in both Australia and South America only in the northernmost portions. *Rana* arose apparently in lands north of Australia and South America. It evidently entered Papua during the Tertiary period when Papua and Australia had become permanently separated. It probably arose in the Old World and entered America by way of the Siberia–Alaska route in Tertiary times reaching Central America before the Isthmus of Panama was formed (mid-Pliocene) and after the Cretaceous East Pacific land-strip had so changed as no longer to form a route to South America. Since the mid-Pliocene neither *Rana* nor *Bufo* has used the Isthmus with freedom for southward migration from Central America, for *Opalina* parasites, abundant in them in Central America, have not crossed the Isthmus. *Rana* carries both *Cepedea* and *Opalina*, especially the latter. It is a most vigorous genus and enters all lands to which the way is open. It probably reached Papua late in the Tertiary and from there passed by some accidental circumstance, very recently, across the narrow channel to the northernmost tip of Australia, Cape York, and has not been there long enough to spread to the south. It is abundant in North America and well represented in Central America. It seems strange that since the middle Pliocene, when the Isthmus was formed, it has not used it more freely for a bridge to South America. Only one species, *R. palmipes*, and possibly two other ranids, *Prostherapis* and *Phyllobates*, got across, spreading only a little way southward.[8] A geologic period and a half, Pleistocene plus late Pliocene, would seem enough to allow many ranids to cross southward, but neither the ranids nor *Bufo* have made use of this bridge, except for the one *Rana* and perhaps the two ranids mentioned. We know that *Bufo* did not cross going southward, for its opalinids are not found south of the Isthmus. At the same time that all bufos and almost all ranids were refusing to pass southward over the Isthmus of Panama, hylas and leptodactylids in abundance were going northward across this bridge. So far as I can see we have no data from hosts or parasites or geologic conditions or climatic influ-

[8] It is likely that *Prostherapis* and *Phyilobates* entered South America with *Dendrobatis*, coming from Africa by a trans-Atlantic route (fig. 143, *a*).

ences to suggest reason for this strange discrepancy between northward and southward migration. That pressure from overpopulation, which may have been greater in the south, could adequately account for this difference seems to me improbable. Note that the dry Sonoran region apparently was a deterrent to migration of moisture-loving Anura. It was effective in stopping the northward movement of leptodactylids, but the hylids swarmed by. But that does not solve the question of the bar to southward migration, for the frogs

FIGURE 155.—Geographic distribution of the Ranidae. *Rana* occurs in all the stippled areas and in the blackened areas, except Madagascar and the Seychelles, but in South America its only representative is *R. palmipes*. Other genera than *Rana* occur in the areas indicated in black. The occurrence of *Ceratobatrachus* in the Solomon Islands is indicated by a black cross.

and toads, with their Opalinas, got by the desert going southward in numbers and were then stopped at the Isthmus.

Ranids other than *Rana* possibly came from eastern Asia-Malaysia to tropical America by way of the circum-Pacific land-strip, in the Cretaceous period, the route used by *Bufo*, though a trans-Atlantic route is more probable. They did not bring *Opalina*, which is not now in South America.[9] For the same reason *Rana* was not in this migration. *Rana* and *Opalina* apparently were in Asia-Malaysia too late to avail themselves of the Cretaceous Pacific land-strip. When, in the Tertiary period, they reached North America by the then opened Siberia-Alaska route, access to South America was shut off by interruption of the land-strip north of South America and, as the Isthmus of Panama was not yet formed, the way to further southward passage was barred.

[9] Since this was first written, knowledge of certain facts makes it questionable, e. g., Opalinas are said to be in South America (Carini, 1937).

Opalina is in tropical Africa, Madagascar, and India. *Rana* is in, but not abundant in, tropical Africa and is in India and Ceylon but not in Madagascar. This suggests that *Opalina* evolved in some form other than *Rana* in Lemuria (Indian Oceanland, including Madagascar, Seychelles Islands, Ceylon, and southern India) after Lemuria and Africa separated in the middle Cretaceous period, and that when it entered Asia proper in the Tertiary, *Rana* had evolved and was ready to serve as migrating host for *Opalina*'s trip to North America by way of Siberia and Alaska. The absence of *Rana* and *Opalina*

FIGURE 156.—Geographic distribution of the Dendrobatinae.

from Australia dates their origin later than the beginning of the Cretaceous. The indication, therefore, is that they arose in the Eastern Hemisphere, in lands north of Australia and separated from it, that is, in Ethiopia-Lemuria during the early Cretaceous. By the middle Tertiary they had a land route to Asia-Malaysia and on to North America, but not to South America. They were too late to catch the Cretaceous circum-Pacific land-strip. Late in the Tertiary there were transient connections with Papua, and *Rana* took advantage of this, but *Opalina* and for that matter *Cepedea* have not been found in Papua.

Of the Ranidae other than the Raninae: *Ceratobatrachus*, from the Solomon Islands, seems a late offshoot from the Raninae; the Dendrobatinae (northern Neotropics) including *Mantella* (Madagascar) suggest spread across the Atlantic (fig. 156). The *Zelleriella* parasites of the former are a late adoption from their American neighbors. The Opalinas of the Madagascar Mantellas were probably adopted

from ranids, or vice versa, in early Cretaceous times. The classification of *Cardioglossa* is doubtful and its opalinids are unknown.

The Ranidae and their Cepedeas and Opalinas may have developed in either Ethiopia or Lemuria. Apparently the Protoopalinas of subgeneric group VIII (ancestors of *Cepedea*), found now in eastern Asia and Malaysia, passed from Asia-Malaysia to Lemuria. While *Cepedea* was evolving in ranid hosts and later giving rise to *Opalina* in the same hosts, the southward spread to Ethiopia may well have been in progress.

BUFO (Fig. 157)

The absence of *Bufo* from Madagascar and the Seychelles and from Australasia including Papuasia indicates (1) origin in Asia in Cretaceous times (no longer in communication with Australasia and Lemuria); or (2) origin in Ethiopia later than the early Cretaceous, after Ethiopia and Madagascar had separated; or (3) origin in South America, probably the northwestern highland region. Origin in Asia in the Cretaceous would leave the way open to South America by way of the circum-Pacific land-strip, but if *Bufo* carried *Cepedea* with it there would be certain difficulties, to be discussed later. If it arose in northwestern South America, the same circum-Pacific land-strip could have carried it to eastern Asia. If it arose in Ethiopia this must have been after the isolation of Madagascar in the middle Cretaceous. *Bufo* did not enter South America from North and Central America after *Rana* and its Opalinas were in these northern lands, or it would have carried *Opalina*, if indeed *Rana* itself would not have accompanied it, and neither *Opalina* nor *Rana* is in South America, except one species of *Rana* (*palmipes*), which seems to be a late Pliocene or Pleistocene immigrant.

A little discussion of the origin and distribution of *Cepedea* is necessary at this point. It is found today throughout the earth where climate is suitable, except in (1) Australasia, including Papua and New Zealand, (2) northeastern United States, and (3) northern South America (probably merely not yet reported here). *Cepedea* evolved from *Protoopalina* through species resembling those now found in Asia-Malayasia (*P. formosae*, *P. quadrinucleata*, *P. axonucleata*). This would seem to indicate Asia-Malaysia as its place of origin. If so, how did it reach Lemuria? No *Protoopalina* is today known from lands reputed to have been once a part of Lemuria (Madagascar, the Seychelles, Ceylon, southern India), except one species in a gastrophrynid from just about the boundary line between southern India and northern India. Similarly *Bufo* is not known from southern Lemurian lands (Madagascar, the Seychelles).

The data known seem to fit the following hypothesis: (1) Origin of *Cepedea* in Asia-Malaysia or Lemuria, before *Bufo* was present there, perhaps in the latest Jurassic or earliest Cretaceous, when

Lemuria and Malaysia were united but Australia and Malaysia were not; (2) the development of *Bufo* in eastern Asia, from some archaic bufonid already having *Cepedea;* (3) *Bufo* passed with its Cepedeas via the Cretaceous circum-Pacific land-strip to Ecuador and later it entered North America in the Tertiary when the land-strip fused with western North America, and its southern portion, between North America and South America, became interrupted; (4) either then or a little later *Rana* with its Opalinas had reached North America by way of Siberia and Alaska and *Rana* and *Bufo* each gave the other of their opalinid parasites, *Rana* giving *Opalina*

FIGURE 157.—Geographic distribution of *Bufo*.

to *Bufo* and *Bufo* giving *Cepedea* to *Rana;* but, the old route to South America being interrupted and the new isthmian route not yet being formed, neither *Rana* nor *Bufo* entered South America from the northern continent after their interchange of parasites. The one paleogeographic feature of this hypothesis that is possibly new is the suggestion that there was a brief time between the Jurassic and Cretaceous periods when Lemuria and Asia-Malaysia were united and Asia-Malaysia and Australasia were not. This involves only slight modification of conclusions by Arldt and others.

Bufo is a most hospitable host, accepting any opalinids it meets. It bears all kinds, its most common guests in any region being the ones most prevalent in that environment.

Bufos, not found in Madagascar and the Seychelles, probably did not enter Africa until the late Tertiary, coming southward from Euro-Asia and reaching Ethiopia by way of the Nile Valley. (*Rana* may have taken the same route at the same time.)

There are some features of the distribution that deserve the emphasis of special mention: (1) No multinucleate opalinids occur in Australasia; (2) *Cepedea* does not occur in eastern North America; (3) *Protoopalina* also is lacking in this area; (4) *Protoopalina* is wholly, or almost wholly, absent from lands once parts of Lemuria; (5) *Zelleriella* has never been in the Eastern Hemisphere north of Australasia. Reasons for these conditions can be given: (1) Multinucleate opalinids evolved in the Northern Hemisphere too late to find any route to Australasia; (2) no Anura, apparently, have crossed westward from Europe to America, the Greenland-Labrador strait not having been bridged, at least at any time when climatic conditions favored the presence of anurans in that region; (3) a far from wholly satisfactory reason for the absence of the primitive genus *Protoopalina* from Lemurian lands may be in the disturbances in land and ocean levels in the Indian Ocean and the final breaking up of Lemuria into several independent portions; (4) *Zelleriella* evolved in Patagonia too late to find a route to the Eastern Hemisphere, except to Australia.[10]

In former studies of the Opalinidae I have traced their evolution in two main lines: (1) From *Protoopalina* through *Cepedea* to *Opalinae latae* and finally to *Opalinae angustae*, and (2) from *Protoopalina* directly to *Zelleriella*. The former named evolution was in the Eastern Hemisphere, from *Protoopalina* to *Opalinae latae*, while the last step, from *Opalinae latae* to *Opalinae angustae*, was in the Western Hemisphere and occurred since the middle Pliocene. The second named evolution occurred in greater Antarctica and later in tropical America. Apparently the evolution of *Zelleriella* took place later than the evolution of *Cepedea* and *Opalinae latae*, but before that of *Opalinae angustae*. There is no indication of the derivation of *Opalina* from *Zelleriella*. In the first line of evolution, the development of multinucleation occurred first and then flattening was emphasized. In the second evolution only flattening is found, multinucleation not developing. No reason is apparent why multinucleation might not have followed rather than preceded flattening. That the phylogeny described is correct is confirmed by the geographic distribution of the several genera in the family: *Protoopalina* and *Cepedea* occur together in the Eastern Hemisphere; *Cepedea* and *Opalinae latae* also; *Opalinae latae* and *Opalinae angustae* occur together only in North America, excepting the late emigrant to Euro-Asia, *O. obtrigona*. On the other hand, *Zelleriella* and *Opalina* occur together only in southern North America which was invaded by *Zelleriella* long after *Opalina* had been evolved in Asia.

There is probably no group of organisms known in which the course of their evolution is more clearly, if as clearly, shown than it is in the

[10] Since this was first written, knowledge of certain facts makes it questionable, e. g., Zelleriellas are said to be in China (Nie, 1935).

Opalinidae. Comparative anatomy, comparative development, and geographic distribution combine to prove the phylogeny beyond reasonable doubt. But while this cannot be gainsaid, some of the hypotheses as to places of origin, times of origin, and routes and times of distribution of the parasites and of their hosts stand on a different basis. Some of the conclusions as to the latter are assured, but others are not. If we grant that the prevalent hypotheses of paleocartographers are correct, the suggestions as to the origins of anurans and opalinids seem to combine the available data in the most acceptable way. But paleogeography remains subject to revision. Realizing this, I have been surprised to find that the data as to anurans and opalinids fit so well the conclusions of Arldt and Schuchert and other paleogeographers. The only disagreement is that the data here studied seem to demand a connection at some time during the Jurassic or early Cretaceous period between Asia-Malaysia and Lemuria. It is the ability of paleogeographic data and conclusions from them to fit such data as the Anura and their parasites present that is one of the chief evidences of the probable accuracy of the paleogeographic conclusions.

But my chief interest in these studies is not in the conclusions established, but rather in the method of gathering and using concomitantly data from organisms and their parasites. This method should be used for very many groups of animals and plants and their parasites, and should always be in the thought of anyone monographing any group, to see if significant data are forthcoming. It will prove a major tool in reconstructing the ancient world and its faunas and floras. It is unfortunate that the paleontological data for the Anura are so scant. Except for this regrettable lack of fossils, the Anura and their Opalinidae give an almost ideal complex of significant phenomena. Some of the many groups having a distribution that is of especial interest as to the question of routes for eastward and westward dispersal in the Southern Hemisphere are the coeciliid, the characinid, and the cichlid fishes and the lungfishes, the ostrichlike birds, and the craneflies. Studies of the parasites of any of these southern groups might well give clinching evidence like that from *Zelleriella* and the South American and Australasian families Leptodactylidae and Hylidae (see Metcalf, 1923a and b; 1928c).

One further consideration should be noted—that the Pleistocene glacial period came too late to be of much significance in connection with the evolution and distribution of the Anura and their Opalinidae, most of this evolution having occurred long before Pleistocene times.

SPECIATION IN OPALINIDAE

The problem of speciation is fundamentally the same as that of evolution. The processes of speciation are different for various groups of organisms. Some species have been formed by sudden and extensive

change from the ancestral condition. Such species are sometimes called sports. Numerous examples are known. One of the best known is that of the sudden appearance of hornless (mulley) cattle, a mutation worthy of recognition as of specific rank. But the distinction between the gradual development and summation of small divergencies and the sudden appearances of major differences is not the only one of interest. The degree to which natural selection affects the development of divergent organisms also is of importance. Speciation of Opalinidae has two noteworthy features: First, species in this family do not arise through the sudden appearance of markedly divergent individuals. This is indicated by the fact that species often so grade into one another as to make it well-nigh impossible to define boundaries between species. Second, natural selection has been less influential in the evolution of the Opalinidae than in the evolution of very many families. This is evidenced by the same phenomena of intergrading species, the struggle for existence not having destroyed the intergrades, but all persisting in apparently equally favorable relation to the environment. We see, then, that the Opalinidae diverge by origin of slight differences and that the slightly divergent forms, having appeared, are unusually free from the action of natural selection.

This freedom from control by natural selection is due to two factors: First, to the fact just mentioned that the divergences arising are very slight and so do not much, if at all, influence success in the struggle for existence. The several slightly diverse forms are all equally successful. The second factor is that the parasites live such secluded lives in so uniform an environment that they escape the stress of life; also there is no diversity of environment to provide peculiar conditions into which specially adapted organisms might fit. Such divergent evolution, speciation, as has occurred in the Opalinidae is, therefore, due less to natural selection and more to the nature of the animals themselves than in most other families. In the Opalinidae the internal factors of evolution are not prevented by environmental influences from expressing themselves. The Opalinidae are what they are through self-determination to an unwonted degree. On this account their character is self-revealing and not due to molding by external influences.

Of course, there is one great exception to this statement. The Opalinidae are parasites, or, more properly, intestinal commensals, and so much of their character as is in adaptation to life within the intestine of a host is doubtless in response to this major condition of their environment. They live bathed in predigested, nutritive fluid, and probably in adaptation to this condition they have no mouth, no digestive vacuoles, and, so far as we know, no digestive fluids, and

they have no organelles for the capture of prey. In such a secluded habitat they have no need of protective devices, such as trichocysts. No locomotor organs for rapid locomotion are needed in seeking prey or in escaping enemies, and so their cilia remain feeble. There seems to be no use for sense organelles and none seem to be found. With sensation and locomotion reduced to a minimum, the neuromotor organs are not emphasized. The excretory vacuoles in some Protoopalinas, on the other hand, give little indication of being reduced. The simplification of structure in correlation with the simplified life in the secluded habitat and with an abundance of predigested food furnished, itself allows less opportunity for the expression of divergent character, that is for specific differences. Partial removal of the animals from the action of natural selection allows such features as do develop through the outworking and self-expression of their own nature to persist. Natural selection does not suppress such slightly divergent individuals as do arise, and thus the whole family, especially the younger genera and subgenera, have but ill-defined species, *e. g.*, *Zelleriella* and the *Opalinae angustae*. In few, if any, other groups of organisms is there better opportunity to study the almost unrestricted outworking of the tendencies inherent in the organisms themselves.

Study of the family in the light of these considerations shows us that there are a number of such trends in them and their evolution could be described in terms of the outworking and interweaving of these trends. (See Metcalf, 1927a.) Some of these trends seem commonplace—a tendency toward flattening; a trend toward elongation; a trend toward posterior pointedness and even the development of a decided, pointed tail; a trend toward curvature of the body, always in the same direction; a tendency toward developing two types of form in the same species, one slender, the other stocky, the difference being in some cases so great as to have led to mistaking the two types for separate species. The very remarkable trend is toward delay in fission after the nucleus has divided, giving rise first to binucleation and later, by the further suppression of additional divisions, to multinucleation. The habit of delaying fission while characteristic of the whole family is developed only to the point of producing binucleation in the most archaic genus, *Protoopalina*, and in the Tertiary genus *Zelleriella*, while in the Jurassic or early Cretaceous *Cepedea* and in the Cretaceous *Opalina* the habit is emphasized to the point of producing multinucleation.

The Opalinidae, of course, are not the only organisms that show a habit of suppressing fissions. Many plants fail to separate their nuclei by cell walls, but this may not be a comparable phenomenon. Among Protozoa certain genera or larger groups are regularly binu-

cleate, for example, *Arcella*, *Giardia*, the Euciliata. Some amoebae are usually binucleate and some are multinucleate (*Pelomyxa*). Some euciliates are multinucleate in certain phases of their life cycle.

In the genus *Protoopalina* one fission (most species), two fissions (*P. quadrinucleata*), or three or more fissions (*P. axonucleata*) may be suppressed; in *Zelleriella* only one; in *Cepedea* and *Opalina* many. *Zelleriella* arose from some species of *Protoopalina* in which multinucleation had not appeared. It arose in Patagonia or Antarctica where those species of *Protoopalina* that are approaching multinucleation (subgenus VIII) do not occur. These are eastern Asian or Malaysian species. The geographical distribution of *Zelleriella* and its ancestors thus agrees with the absence of any developed tendency toward multinucleation.

We have spoken of a trend toward suppression of one or more fissions. There are other trends in the family—a trend toward flattening, which receives two independent emphases, first, in the formation of *Zelleriella*, second, in the formation of *Opalina*; a trend toward elongation, which likewise received two independent (?) emphases, in the elongated Cepedeas, and in the elongated Protoopalinas; and others. I believe that the whole evolution can profitably be discussed from the standpoint of trends, their occurrence, their origin, their growth, their waning, their disappearance,[11] their independence, their interdependence (see Metcalf, 1927a). We find evidence as to the part of the earth and the geologic time in which appeared emphases upon certain trends: for example, emphasis upon flattening appeared once in southeastern Asia or in Lemuria in the Cretaceous period (*Opalina*), and once in Patagonia at some time between the middle Cretaceous and the middle Miocene, probably during the early Tertiary.

In no group of organisms is there better chance to study the nature of the organisms themselves as expressed in their evolution, relatively undisturbed by pressure of their environment. In the evolution of forms that have left even the fullest fossil record, it is very difficult to evaluate the environmental factors and the internal factors. The relative importance of the two classes of factors may be very different in various organisms. One should, therefore, be very cautious in drawing general conclusions from one group and applying them to another group.

One feature of the evolution of the Opalinidae seems of rather general application to internal parasites. The adaptations to parasitism, if they occur at all,[12] are likely to take place promptly, and the subsequent evolution to be comparatively slow and slight because of the removal of much of the pressure of the environment. We have

[11] Suppression might be a better word than disappearance to use here.

[12] Little structural adaptations to parasitism are observed in *Balantidium* and *Nyctotherus*, which live with the opalinids in the recta of Anura.

seen that the Opalinidae arose in southern, perhaps sub-Antarctic, lands as early as the Triassic period.[13] Some of these archaic forms are living in the same regions to-day and are almost unmodified. During at least part of the Triassic period and all the Jurassic, the Cretaceous, and the Tertiary periods, these primitive species (*Protoopalinae* of subgeneric group I) have persisted. The bell toad parasites (*Protoopalinae*, group II) are practically unmodified after persisting at least since the Jurassic period; and so on for other genera and species for different lengths of time. The widely evidenced general principle that evolution is rapid during periods of environmental change and is slow during periods of environmental uniformity receives added support from the Opalinidae. Evolution may proceed under the influence and control of internal factors, but it is likely to be speeded up when environmental pressure (change) and internal responses are operating together, but under such circumstances it is difficult to give proper relative credit to the two sets of factors.

The stream of protoplasm that was, in the Triassic period, and still is *Protoopalina* has formed many eddies, species, along its course. At least 48 of these eddies, and probably others not yet discovered, have persisted through many millions of years until the present time. Doubtless others have disappeared, but the persistence of such species, eddies, when once formed, is remarkable. Some of these eddies, *Protoopalinae* of subgenus I, arose in the Triassic period; others, subgenus II, probably at the time of the development of the bell toads, which may have been as late as the earliest Cretaceous, but not later, although they were probably of Jurassic origin. Still others, subgenus VIII, were present at a little later period in Asia-Malaysia when *Cepedea* evolved. The species of subgeneric group VI (parasites of *Scaphiopus*) did not form until some time in the Tertiary, perhaps late in the Tertiary. Through all this immense stretch of time from the Triassic into the Tertiary the stream was forming new eddies and occasionally an eddy divided forming two or more (4 in the case of *Protoopalina*, subgenus VI), and when formed they tended to persist, or at least many of them did. The successive eddies diverge from one another by slight increments of difference, the species now found forming a remarkably complete series with no great gaps. Speciation in *Protoopalina* has not been by sporting, by sudden, extensive mutation, but by changes that have been very gradual, almost every conceivable intergrade between the imperfectly binucleate *P. primordialis* and the multinucleate *P. axonucleata* being found. This series shows the changes in line with the trend to multiplication of nuclei. Especially in subgenus VI, very late in this series, the increments of change between species are slight. For further illustration of the very minute increments of change, mutation, by

[13] Probably not much, if any, earlier, for the Anura are not much older.

which the species of Opalinidae diverge from each other, see the genus *Zelleriella* and the subgeneric group *Opalinae angustae*, both of which we have regarded as comparatively modern. When once the adaptations to parasitism had been secured, there is no indication, at any point in the further development, of any speeding up of the processes of evolution.

A REVIEW OF THE LITERATURE OF THE OPALINIDAE SINCE 1923

In 1909 and 1923 I critically reviewed the literature of the Opalinidae. The present review is intended to bring the survey down to date. First let us mention a few papers that were omitted from the former reviews or received insufficient reference.

In 1891, L. and L. Zoya discussed the fuchsinophile plastids (bioplasts) of Altmann, briefly describing those of *Opalina ranarum*.

In 1904, Cobb, not mentioning the opalinids, used parasites to indicate genetic relationships between organisms, much as I (Metcalf, 1928c) used opalinids in discussing paleogeography and geographic distribution. Cobb's interesting paper should be mentioned in that connection.

In 1913, Poche, in discussing the taxonomy of Protozoa, mentions that in opalinids the "generative and morphological nuclei" are not separated.

In 1916, Mavor discussed *Myxidium lieberkühni*, a parasite of European and American pikes, much as Kellogg discussed the mallophagous parasites of birds to indicate descent from a common ancestor. This paper should have been mentioned in Metcalf, 1928a.

Ghosh, 1918, reports three new (?) species of opalinids from India: *O.* [*Cepedea*] *scalpriformis*, *O. plicata*, and *O. triangularis*. (With the exception of the first, the descriptions are too scant to allow specific identification.)

Ghosh, 1920, discusses the cytology of *Opalina* [*Cepedea*] *scalpriformis*, says it is abundant in winter, is comparatively rare at other seasons, that its "chromosomes" [nucleoli] are six in number, that its length is 24–57μ, its greatest width 8–15μ.

In 1920, Tönniges described the mitosis of *Opalina ranarum*, but it was not until seven years later (Tönniges, 1927) that he published the illustrations.

A paper by Chatton and Perard, in 1921, refers briefly to the Opalinidae and to the fact that their period of encystment corresponds to the breeding period of their hosts as significant in connection with the evolution of parasitism. It also mentions the absence of encystment in *Opalina* [*Protoopalina*] *saturnalis*, a rare condition among parasitic ciliates.

Two little notes by Metcalf (1922a and b) call attention to alcoholic specimens of Anura as a source for reasonably well preserved opalinids

and suggest the utility of the Greek word for guest, *ξένος*, in forming names for parasites.

In the same year Kudo (1922) refers to Opalinas from adult *Rana clamitans* and *R. pipiens* as seemingly identical with *O. ranarum*. [*O. ranarum* has not been found in America. Adult *R. clamitans* is but very rarely infected.]

Konsuloff (1922) was inadequately referred to in Metcalf (1923a). His paper is based chiefly upon *O. ranarum* and *O.* [*Cepedea*] *dimidiata;* anisogamy is described, also encystment of quite large multinucleate individuals; division of endosarc spherules is described [confirmed by Horning, 1925]; *O. ranarum* has much-branched excretory canals in the posterior part of the body; the endosarc spherules are called macronuclei [erroneous, see Tönniges, 1927]; the author confirms the opinion that *O.* [*zelleri*] is a form of the species *dimidiata;* he describes many features of the cytology, also extracellular digestion [but gives no evidence]; in the adults, but not in the tadpoles, the opalinids are said to be positively geotactic; endogenous cysts were found within encysted adults; the agamonts hatching from these cysts are said to develop directly into adults with no interpolated sexual process [not shown but not improbable]; the encystment of zygotes is described as normal [the claim being founded, perhaps, on finding binucleate infection cysts, which are quite common]; the multiplication of nuclei of encysted zygotes is described [based perhaps on infection cysts with more than two nuclei, which are not unusual, as many as 12 or more sometimes being found. Cf. Brumpt, 1915. Reinfection cysts in the tadpoles, described by Brumpt, might be formed as early in the growth as the zygote stage]; Metcalf's classification is confirmed; there is no formation of nuclei from chromidia; crystalline excretory granules in the cytoplasm are mentioned.

Hegner, in 1922, reported that on a meat diet the tadpoles of *R. clamitans, R. pipiens,* and *Bufo lentiginosus americanus* [*B. americanus*] decrease the number of their opalinids.

Metcalf (1923a) described about 125 new species of opalinids, mostly from Anura long preserved upon the shelves of the United States National Museum; the geographic distribution of hosts and parasites was discussed, as well as the probable place and time of origin and the times and routes of dispersal with reference to paleogeographic maps of the successive geologic periods from the Triassic to the present; a critical chronological review of the opalinid literature not included in Metcalf, 1909, was given.

The same year Metcalf (1923b) discussed the origin and distribution of the Anura on the basis of their opalinid parasites and the geographic distribution of the hosts and parasites.

Spek, in 1923, described the effects of different salt solutions upon living *Opalina ranarum,* showing that with changes in the medium

there were such changes in the structure of the protoplasm as to suggest caution in conclusions as to normal structure.

Fantham, in 1923, published the first of a series of six papers describing new species of opalinids from South Africa, chiefly from Johannesburg, as follows: 1923, *Protoopalina transvaalensis*, with notes upon *P. xenopodos* and *P. mossambicensis*; 1924, *Opalina sudafricana*; 1927, further notes on *P. transvaalensis* and *O. sudafricana*; 1929, *P. appendiculata*, *P. ovalis*, and *P. caccosterni*; 1930, *P. octomixa*; Fantham and Robertson, 1928, *P. meridionalis*. Measurements and drawings of all these forms are quoted in the taxonomic portion of the present paper.

In 1924, Metcalf called attention to the fact that his *Opalina japonica* had been previously described by Sugiyama and had been given the same name.

Hegner, in 1924, reported that in *Opalina* [*larvarum* (?)] from tadpoles of *Rana clamitans* and *R. catesbeiana* the nuclei are evenly distributed through the cytoplasm and probably control approximately equal masses of cytoplasm; that size of body and number of nuclei are very closely correlated; that by diminution in size of the older nuclei the ratio between volume of nuclei and volume of cytoplasm is maintained; that division of one nucleus (and only one) occurs when the proportion of cytoplasm becomes too great.

Cleveland, 1925, reported that oxygenation at 3.5 atmospheres pressure killed *Opalina* within the host in 18 minutes.

The same year, Larson, Van Epp, and Brooks reported the length of life of *Opalina* outside the host in 8 different liquids.

Horning, 1925, studied *Protoopalina* and described mitochondria and their different forms in all stages of the life cycle, regarding them as persistent, self-reproducing bodies and not as products of metabolism, "though the latter possibility has not been disproved." Synthesis of vegetative granules (storage products) may take place at the surface of the mitochondria.

Gatenby and King, 1925, regard *Opalina ranarum* as a flagellate, because the cilia "enter right into the substance of the organism and take their origin from the peculiar granules, 'blepharoplasts' [endosarc spherules] which exist in very large numbers" [mistaken observation (?)].

Wenyon, 1926, in his fine, 2-volume Protozoology, accepts Metcalf's (1923a) classification of the Opalinidae and gives adequate review of recent literature. He figures [original] encysted *Opalina ranarum* with 1, 4, 6, and 22 nuclei [cf. Konsuloff, 1922, and Metcalf, 1909].

In 1926, van Orden and Nelson reported as follows: One specimen of adult *Rana clamitans* was found well infected with *Opalina*; inoculations of *R. clamitans* with adult *Opalina* from adult *R. pipiens*

(5 inoculations) and *R. palustris* (2 inoculations), and secondary transfers from artificially infected *R. clamitans* (1) and *R. catesbeiana* (1), also inoculations of adult *R. catesbeiana* with adult *Opalina* from adult *R. pipiens* (3) and adult *R. palustris* (3) and from artificially inoculated *R. clamitans* were tried, and upon examination about once a month gave the following results: *R. clamitans*, 5 showed no infection; after inoculation from *R. pipiens* 2 showed good infections after 162 and 174 days; after inoculation from *R. palustris* 1 showed fair infection after 16 days, a second 82 days after secondary inoculation from an artificially infected *R. catesbeiana* showed fair infection. Experiments upon *R. catesbeiana*: 4 showed no infection after inoculation; 1 gave a good culture 35 days after inoculation from adult *R. pipiens*, but none after 71 days; 1 gave a fair culture 105 days after inoculation from *R. pipiens*; 3 inoculated from adult *R. palustris* and 1 secondarily inoculated from an artificially infected *R. clamitans* all were negative, none having established infections.

Ten Kate, in 1926, regarded the system of fibrils, described in detail, as having only a supporting function [an interpretation made doubly improbable by Taylor's (1920) microdissections of *Euplotes* and the destruction of coordination in the beat of the cilia by severing portions of the system of fibrils]. The endosarc spherules are [mistakenly] regarded as macronuclei.

Gourvitsch, 1926, redescribed under the [mistaken] name *O. elongata* n. sp. specimens of *Cepedea saharana* Metcalf from *R. esculenta ridibunda* from Tashkent, Turkestan [see Metcalf, 1927b].

Da Cunha and Penido, 1926, described *Zelleriella piscicola* from a catfish (?) from the Paraguay River.

Tyler, 1926, stated that *Opalina* may live 25 days without change of medium, in modified Pütter's fluid used according to Konsuloff, 1922.

Metcalf, 1926: In the tadpoles of the hosts the opalinid parasites start their development in the condition of *Protoopalinae* of the most primitive subgenus and pass through larval stages corresponding to the phylogeny of the family until they reach their definitive character. *Zelleriella* passes through a *Protoopalina* stage; *Cepedea* through successive *Protoopalina* stages, including at least subgenera I and VIII of the present paper; *Opalinae latae* add to this series the broad, flat stage characteristic of the adult; the *Opalinae angustae* pass through all these stages, then become definitively narrow, thus confirming the course of the phylogeny as I had before outlined it.

Klein, 1926, described and figured a very primitive "silver line system" in *Opalina ranarum*. The basal granules of the cilia and the longitudinal striae impregnate with the silver, the former being blacker.

Bhatia and Gulati, 1927, reported opalinids as follows from India: From *Rana tigerina*, *Opalina coracoidea lahorensis*, new subspecies, new host, new locality; from *R. cyanophlictis*, *O. ranarum*, new locality; from *R. hexadactyla*, *O. lata*, new host, new (?) locality; from *Bufo melanostictus*, *Cepedea metcalfi*, new species, *C. punjabensis*, new species, *C. sialkoti*, new species.

Metcalf, 1927a, discussed the evolution of the Opalinidae from the standpoint of certain trends (to flatness, to elongation, to posterior pointedness, to delay in division of the body, to delay in completion of mitosis), phenomena so distributed among the subdivisions of the family as to involve either repeated fortuitous appearance of these characters, a thing not to be believed, or trends resident in the germplasm. These conditions are compared with similar phenomena in the Ophryoscolecidae and the Salpidae, and the relation of trends to evolution is discussed.

Tönniges, 1927, described mitosis in *Opalina ranarum*, bringing it into line with that of other organisms. Eight "macrochromosomes" [nucleoli] are described, 24 "microchromosomes" [chromosomes]. The "nucleolus" disappears during mitosis [against Metcalf, 1909]. Amitotic division is described and figured by the author. Figures of mitosis and of direct division, prepared in 1897, are here published for the first time.

Metcalf, 1927b, points out that Gourvitsch's *Opalina elongata* is Metcalf's *Cepedea saharana*.

Lavier, 1927, describes four infections of *Protoopalina nyanza* from a lizard, *Varanus niloticus*, from the shores of Lake Victoria Nyanza. The description is quoted in the present paper.

Sokolska, 1927, reported for *Opalina ranarum* the Golgi apparatus and mitochondria as disk-shaped bodies strewn through the cytoplasm, consisting of a lipoid membrane and a weakly staining globule upon it [seeming from the illustrations to be the endosarc spherules], and also a line of granules down the axis of each cilium, figured and interpreted as mitochondria.

Larson, 1928, reported rearing *Opalina* in Cleveland's, Pütter's, Locke's, and Ringer's solutions, adding egg albumin or blood serum [not predigested], Pütter's fluid plus blood serum seeming the best [worth retesting to see if opalinids do use undigested food]. Adding a bit of rectal wall and subculturing every day or every second day make it possible to maintain a culture a month or more.

Metcalf, 1928a, discussed with the aid of their *Protoopalina* parasites the origin and spread of the bell toads, Discoglossidae. *P. stejnegeri*, new species, from *Ascaphus truei* is described.

Harrison, 1928, discussed host-parasite relations including those of the Opalinidae and their anuran hosts. Reference was made to

Metcalf's studies on the geographical distribution of the opalinids and their hosts.

Larson and Allen, 1928, reporting again upon rearing of *Opalina*, said that 80 out of 166 specimens of *Rana pipiens* were "sufficiently heavily parasitized for use" with *Opalina obtrigonoidea*.

Reichenow, 1928, reported that the granules of the nuclei give positive reaction with Feulgen's stain, while the endosarc spherules do not. [There is evidence, some of it unpublished, that nuclear structures which at times react strongly to Feulgen's stain, under other conditions do not.]

Swarzewsky, 1928, compared sexual and presexual phenomena in *Spirochona elegans* with those in Opalinidae and euciliates in general.

Metcalf, 1928b, discussed, in the light of Boveri's hypothesis as to the fundamental nature of cancer (Boveri, 1914), certain abnormal individuals of *Opalina obtrigona*, *Zelleriella*, and *Protoopalina caudata*.

Metcalf, 1928c, discussed, before the American Society of Parasitologists, parasites and the aid they give in problems of taxonomy, geographical distribution, and paleogeography. (This is but an abstract. See Metcalf, 1929a, for full publication.)

Thompson and Robertson, 1929, made no reference to the Opalinidae, except showing a good, original microphotograph of *Opalina ranarum* and making the [erroneous] statement that *Opalina* occurs in nearly every frog.

Doflein and Reichenow, 1929, give in the third volume of their textbook a full account of recent work by numerous students, with some original drawings. [Metcalf is erroneously reported as having described a *Zelleriella* stage in the development of *Opalina;* see p. 1164]. The nuclear nature of endosarc spherules is opposed.

Van Overbeek de Meyer, 1929, after a review of the literature of the Opalinidae, reported: (1) *Opalina* cysts from adult frogs develop in two ways, one with and the other without interpolation of the sexual process [this is probable but not yet established by sufficiently guarded, critical experiment]. (2) The term "ectoplast" is preferred to ectoplasma. (3) There are no neurofibrillae in the inner layer of the "ectoplast." (4) The basal granules of the cilia arise *in situ* from a fibril of ectoplast and independently of the nucleus. (5) A fibrillar system develops temporarily as a network of supporting elements. Its origin depends upon the state of development of the plasma. (6) The cytoplasm shows during the growth of the animal a definite development by which the number and size of spaces in the plasma slowly increase up to almost the adult condition. After this the cavities become again smaller and fewer, while the plasma connections between become thicker, the plasma thus becoming again compact. During encystment this process is reversed but goes more

rapidly and with less evident stages. (7) In the adult there is no pellicula, but instead a tough, cheeselike condition of the plasma itself, which serves to retain form of the body. (8) Drying causes prompt gelation of the plasma. (9) There appears to be a centrum for cilia movement at the anterior end of the body, apparently in the growth zone of the cilia. (10) The ectoplasmic and entoplasmic inclusions seem to be stages of one and the same secretion process. (11) Bhatia's doubtful report of isogametes is quoted with approval [not confirmed by recent students]. (12) The fibrils associated with the rows of cilia on the upper side of the body are not continuous with those on the under side, but the two sets meet and join, at irregular intervals, a coarser transverse fiber at the anterior edge of the body. The paper contains important details of cytology and development not here mentioned. The author does not call attention to it, but one observes the fact that, in his careful detailed drawings, no single instance of apparent fission of endosarc spherules is shown [see Horning, 1937] [many statements of de Meyer need confirmation.]

Metcalf, 1929a, published in full the paper abstracted in Metcalf, 1928c. Cobb's paper, 1904, and Mavor's, 1916, should have been included in the discussion.

Metcalf, 1929b, pointed out that the Opalinidae are pivotal forms, instructive, first, as to the origin of the Euciliata, and, second, as parasites that, along with their anuran hosts, lend themselves with peculiar advantage to host-parasite studies of paleogeography.

Metcalf, 1929c: The occurrence of a reputed leptodactylid, *Heleophryne*, in South Africa, while the home of this family is in South America and Australia, is discussed as a bit of evidence that a South Africa–Patagonia connection was present and that it persisted until somewhat later than is usually thought, that is, into the Cretaceous period, or possibly the Tertiary, provided that *Heleophryne* is, indeed a leptodactylid.

Higgins (1929) quoted Metcalf's observations (1926) upon a succession of larval forms in the development of *Opalina larvarum*, forms that repeat the phylogeny of an *Opalinae latae*, and compares with these phenomena the very divergent forms of *Nyctotherus cordiformis* found in larvae of American frogs and toads.

Haye, 1930, quoted with disapproval van Overbeek de Meyer's [mistaken] suggestion that the "excretory canals" [not canals] of *Cepedea dimidiata* are artifacts. Haye found none present in *Opalina ranarum* [cf. Konsuloff to the contrary, 1922] He [mistakenly] calls the endosarc spherules macronuclei. The excretory apparatus he attributes to degeneration [error, though in individuals kept under unfavorable conditions it may increase in size]. He [mistakenly] interprets the cytomicrosomes as bacteria.

Konsuloff, 1930, discussing the nuclear nature of the endospherules, said that these disk-shaped bodies are macronuclei, are permanently formed structures, have an evident, thick membrane, divide without chromosome formation, disappear during the sexual process to reappear later. He [erroneously] says that de Meyer and also Metcalf would have accepted the macronuclear nature of the spherules if they had believed that the spherules divide, a conception for which Konsuloff and especially Horning (1937) give evidence. [Not so. The macronuclei of euciliates are true nuclei, derived from micronuclei. This cannot be true of the endospherules of opalinids. They may possibly be composed of, or derived from, metabolic chromatin, as Metcalf, 1909, suggested (cf. Reichenow, 1928), but they are not metamorphosed nuclei.]

At the Christmas meetings of the American Society of Zoologists in New York City in 1928 Kofoid and Dodds reported work upon *Opalina obtrigonoidea* and *O. virguloidea*. The abstract of their revolutionary paper is quoted here. "Mitosis in the two species has been studied. At nuclear division an extranuclear centrosome divides and the daughters migrate to the poles of the elongating nucleus, forming an extranuclear paradesmose, as in the flagellates. Each nucleus has a slender rhizoplast running from the centrosome on the periphery of the nuclear membrane to a basal granule—in reality the blepharoplast—from which the flagellum, the so-called cilium, emerges. From the blepharoplast another rhizoplast runs down to the so-called endoplasmic spherules, which are interpreted by us to be parabasal bodies. The unit of the neuromotor system in the species observed thus consists of the following parts: the flagellum, its blepharoplast, parabasal body and its rhizoplast, and, if attached to a nucleus, the rhizoplast running from the blepharoplast to the centrosome on the nuclear membrane. The blepharoplasts are arranged in spiral lines and the parabasal bodies are distributed below them less clearly showing the spiral arrangement. The neuromotor units are thus considerably in excess of the number of the nuclei. A similar relation has been evolved in a number of multicellular types of flagellates found in the termites, in which, as in the genus *Calonympha*, there are more neuromotor units than there are nuclei. The neuromotor system and the type of mitosis in the opalinid Protozoa are clearly homologous to that of the flagellates. In the light of these facts, it is logical to transfer the Opalininae from the Ciliata to the Flagellata." [Publication in full must precede adequate criticism, but we may note: (1) that no other students have shown centrosomes, even after prolonged search by a great variety of techniques; (2) that the appearance of a longitudinal fiber upon the caryotheca of the dividing nucleus is occasionally seen, especially in the living animal, but prolonged

attempts to demonstrate this in stained material have failed; (3) that connection between basal granules and nuclei has not been demonstrated and if present would be exceedingly difficult to trace among the very numerous fibrils that permeate the cytoplasm in every direction; (4) that connection of endoplasmic spherules to the neuromotor system has not been seen by others, and Horning's and also Scott and Horning's studies indicate that the spherules are connected with a structurally and functionally different set of organs; (5) that the basal granules ("blepharoplasts") are much more numerous than the endoplasmic spherules ("parabasal bodies") and that there is no comparable spiral arrangement of the two sets of structures; (6) that relationship of both Protociliata and Euciliata to the Flagellata is altogether probable, and it seems perhaps possible that there may be a bit closer relationship between Protociliata and Trichonympha, but comparison of Ciliata and Protociliata is closer and more significant.]

Noble, 1931, referred to the evidence from *Zelleriella*, cited by Metcalf, that this genus and its leptodactylid hosts were never in northern continents and so must have passed between South America and Australia by some Southern Hemisphere route, and he demurs, saying, "It may well be, however, that the northern opalinids were not in existence at the time the present southern opalinids were being carried south by whatever species they happened to parasitize at the time" [a sentence whose meaning in this connection I cannot solve].

Hegner (1931) reported on August 29, 1930, to the Helminthological Society of Washington that of 10 adult *Rana clamitans* from Mount Desert Island, Maine, none were infected; that the opalinids are lost during the metamorphosis of the tadpoles, between the stages showing only hind legs and the stages with all four legs evident. Young green frogs could not be infected by mouth or by rectum with opalinids from green frog tadpoles [cf. van Orden and Nelson, 1926]. Opalinids were found in tadpoles of tree frogs [species not mentioned] during all stages of metamorphosis and also in the "young adult" tree frogs. He expresses the opinion that apparently some digestive secretion peculiar to the green frog appears during metamorphosis that renders the rectum of this species unfit as a habitat for opalinids.

Merrill, 1931, in a lecture before the Washington Academy of Sciences, discussed the relations of Philippine biota to the faunas and floras of Malaysia and Australasia and showed frequent changes in connections between lands in these areas during and since the Pliocene period. These affected the distribution of the opalinids and their anuran hosts and are referred to in the present paper.

Richardson and Horning, 1931, described, with adequate figures, "mitochondria" in *Protoopalina*, "together with associated, synthe-

sized vegetative granules and Golgi bodies, as evidenced by their behavior, morphology and staining reactions."

De Mello, 1931, described parasitic infusorians in *Rhacophorus maculatus* in Nova Goa, among them *Opalina virgula* Dobell, *Cepedea longa* Bezzenberger, and *Cepedea thiagi* de Mello.

Scott and Horning, 1932, reported that Opalinas from the rectum of *Rana pipiens* [doubtless *O. obtrigonoidea*] when imbedded in paraffin and microincinerated according to the technique of Policard, and then examined by dark-field illumination, retained their distinctive cytoplasmic morphological characters in the ash deposited in the same topographic relations as those shown by the cell-inclusions in stained specimens. The coarse vegetative granules, the myonemata and the cilia were "perfectly preserved." The more or less abundant chromatin, however, left little or no ash, in marked contrast with Scott's findings with amphibian and mammalian nuclei.

Chen, 1932a and b (abstracts), described mitosis in a species of *Zelleriella*. He found that the behavior of chromosomes is essentially the same as that in the Metazoa and Metaphyta. Among the 24 chromosomes found in this species the six shortest ones could be recognized in every dividing nucleus. (See comments by Metcalf in Chen, 1932a.)

Patten, 1932a, a preliminary note.

Patten, 1932b, made a detailed study of the endoplasmic bodies in *Opalina ranarum*. The endospherules in this opalinid are said to be somewhat flattened disklike structures or dumbbell-shaped forms. Usually in sections cut parallel to the flattened surface of the organism these bodies are rounded or rather irregular in shape, while in sections cut transversely to the flattened surface they are mostly rod-shaped or frequently drawn out into dumbbell-shaped forms, while in oblique sections every form may be seen with gradations from rod-shaped to irregular shapes. Richardson and Horning had previously considered that two types of bodies are present: The irregular, faintly staining granules—the vegetative granules—and the rod-shaped or dumbbell-shaped mitochondria. According to Patten there is but one class of body in *Opalina ranarum*. The rod and irregular bodies are thought to be but two aspects of the same body. She furnished additional evidence supporting her view in that the dumbbell forms (mitochondria of Horning), as well as the irregular forms, are well shown by alcoholic fixatives that normally dissolve the mitochondria. Living material was also studied. It was her belief that these endoplasmic bodies are probably neither Golgi bodies nor mitochondria. She does not believe that they are identical with the macronuclei of other ciliates, as Konsuloff and others claimed. She was inclined to believe that the endospherules may be concerned with

storage or synthesis of food materials. In addition to the bodies mentioned above, she found very fine granules in the endoplasm, which are always spherical and very similar in size, considered by her to be mitochondria.

De Mello, 1932, described among other opalinids from Malabar one new species: *Cepedea subcylindrica* from *Bufo melanostictus*. [The description is too scant.]

Nie, 1932, reported the presence of four species of opalinids in *Rana limnocharis* Gravenhorst, found in Nanking, China, three of which were new: *Protoopalina limnocharis*, *Opalina undulata*, and *O. acuminata*.

Carini, 1933b and c, described two new species of *Zelleriella* from Brazil: *Z. falcata* in *Engystoma ovale* and *Z. cornucopia* in *Leptodactylus ocellatus*.

Ivanic, 1933, described mitosis in *Cepedea dimidiata* Stein in *Bufo vulgaris* Laurenti. *C. dimidiata* has numerous typical vesicular nuclei and disk-shaped bodies. The vesicular nuclei have a membrane, a plastin karyosome, and a ball of linin on which are scattered chromatin granules. The chromatin granules are arranged in 6–8 longitudinal rows [chromosomes] on the spindle and divide. According to Ivanic the "macrochromosomes" are only clumps of plastin karyosome; the "microchromosomes" are the rows of chromatin granules on the spindle. Ivanic believes that the discoid bodies are small nuclei with the same structure and behavior in division as the large nuclei [probably a mistaken observation]. The discoid bodies divide by mitosis [an observation which needs to be confirmed] and are present throughout the life cycle.

Zingher, 1933, reported the presence of fat inclusions in *Opalina ranarum*. He noted that in the smaller individuals the fat bodies are much larger and more numerous than those in the larger specimens. In the larger specimens the fat bodies are more uniformly distributed, whereas in the smaller specimens there is an accumulation of these fat bodies at one end of the body. In the opalinids found in tadpoles these fat bodies are entirely lacking.

Stabler, 1933, was the first to identify correctly the endamoebae parasitic in the opalinids that had previously been erroneously thought to be a new genus—"*Brumptina*" by Carini (1933a). Stabler found both trophozoites and early cystic stages of this *Endamoeba* in the Zelleriellas from Arizona and Panama. The amoebae rest in small pockets in the endoplasm of the opalinid. In any one opalinid, all the amoebae were found in very nearly the same stage of development, i. e., all cysts or all trophozoites. [Stabler's report was independently confirmed by Carini and Reichenow, 1935; the latter two investigators described the amoebae in greater detail]. This is but an abstract. See Stabler and Chen, 1936, for full publication.

Metcalf, 1934a, gave a summary of the results of his concomitant studies of the taxonomy and geographical distribution of the Anura and their opalinids. Detailed data may be found in the present paper.

Ivanic, 1934a, stated that the endospherules are true nuclei. He found that in atypical infection cysts that lack vesicular nuclei the endospherules develop into vesicular nuclei [this report needs confirmation].

Ivanic, 1934b, stated that the vesicular nucleus of *O. obtrigona* carries on the linin network one or more plastin masses in addition to the chromatin granules. The chromosomes develop from a spireme at the time of nuclear division. The plastin karyosomes are distributed more or less irregularly to the daughter nuclei, but they may also divide into daughter karyosomes. The persistence of plastin material through the entire division process indicates that it is promitotic in origin. The endospherules described by Mayer in *O. ranarum* as being connected with nutritive processes are actually small vesicular nuclei. [Probably an erroneous belief.] The author believes that these can arise "de novo" and grow to normal size. [This needs confirmation.]

Valkanov, 1934, was of the opinion that the so-called "macrochromosomes" and nucleoli are homologous and that they are to be considered as trophic elements. He also believed that there is only one type of nuclei in the opalinids against Konsuloff and Ivanic.

Nie, 1935, described the opalinids found in the amphibians from Nanking, China. Among others the following new species and subspecies were described: *Protoopalina caudata microhyla* in *Microhyla ornata; P. pingi* in *Rana plancyi* Lataste; *Zelleriella orientalis* in *Microhyla ornata* Boulenger; *Opalina cheni* in *Kaloula borealis* Barbour; and *O. obtrigonoidea* forma *lata* in *Kaloula borealis* Barbour.

Wenrich, 1935, showed that host-parasite relationship in the Opalinidae is not specific, since each of the four genera of opalinids has species distributed through four families of anurans and two of the genera of these ciliates also have species in the tailed Amphibia. He also cited the experimental cross infections of opalinid hosts made by Metcalf (1909), indicating that there is no rigid host-parasite specificity.

Carini and Reichenow, 1935, described the endamoebae parasitic in *Zelleriella* from South America. The amoeba trophozoites measure 8–14μ, the cysts 8–12μ. The structure of the cysts and trophozoites of amoebae was described in some detail, and resemblance of this amoeba to *Endamoeba ranarum* was pointed out. They believe that this amoeba is either identical to *E. ranarum* or a species (or race) derived from it.

Carini, 1935, reported the presence of *Opalina* in Brazil.

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Chen and Stabler, 1935, found that the endamoebae parasitic in the opalinids have a very wide geographical distribution. This is but an abstract. See Chen and Stabler, 1936, for full publication.

Stabler and Chen, 1936, described in some detail the endamoebae parasitic in the opalinids. Trophozoites and cysts of *Endamoeba* were described in detail, variations noted. The endamoebae seem to produce no serious effect on the opalinids, as the latter swim actively in the saline solution and undergo binary fission even though heavily parasitized. The endamoebae in a species of *Zelleriella* from Chile were found invaded by a *Sphaerita*-like organism. No specific name was given to this *Endamoeba*, which closely resembles *E. ranarum*.

Chen and Stabler, 1936, found that the endamoebae parasitic in the opalinids have a very wide geographical distribution, being found in Egypt, China, Ceylon, the United States, Panama, Brazil, Uruguay, and Chile. Different species belonging to all the four genera of the family Opalinidae have been found parasitized by the amoebae. The amoebae were also found in cysts of opalinids, thus constituting an important method of transmission of amoebae from adult anurans to tadpoles.

Hegner, 1936, found that certain flagellates in the frog seem to live longer than certain ciliates, after the host is dead. *Opalina* lived for at least 4 days after the anuran host (frog) had died.

Ivanic, 1936, described the mitosis in *Opalina ranarum* and in *O. obtrigona*. According to him the resting nucleus contains "plastin" in one or more pieces. This "plastin" material may partly disappear during mitosis and may be irregularly distributed to the daughter nuclei. He believes [correctly] that there are no "macrochromosomes" and "microchromosomes" but only one type of chromosomes derived from the chromatin granules in the resting nucleus [no reference was made to the work of Pfitzner on *Opalina ranarum* in 1886 and to Tonniges' work on the same species in 1927].

Chatton and Brachon, 1936, on the basis of the arrangement and fate of cilia lines of opalinids during division suggested that opalinids are intermediate between the flagellates and ciliates.

Chen, 1936a and b, gave a detailed account of mitosis in *Zelleriella*. He reported that the behavior of chromosomes during mitosis is essentially the same as that found in multicellular organisms. He found, for the first time for opalinids, (1) that the chromosomes are of different sizes and shapes and can be individually recognized; (2) that there are two chromosomes of each size and shape, indicating diploidy; (3) that the nucleoli are constantly associated with certain portions of certain chromosomes: (*a*) Depending on the subspecies, there may be 4 or 6 nucleoli formed respectively on 4 or 6 (2 or 3 pairs) of the 24 chromosomes; (*b*) the location of the nucleolus is

identical on the members of each chromosome pair; (*c*) the chromosome segment on which the nucleolus is located may undergo considerable structural modification; (*d*) the behavior of the nucleolus-bearing chromosomes during mitosis is the same as that of the other chromosomes; (*e*) when two nucleoli are close together they may fuse, thus giving rise to apparent variation in the number of nucleoli within the species which has been so often reported by other investigators; (*f*) in the resting nucleus the nucleoli are arranged at random and are located at the periphery; these nucleoli were mistaken for chromosomes by many previous investigators; (4) that the so-called "macrochromosomes" in the opalinids are not chromosomes but nucleolar regions of certain chromosomes; (5) that the so-called "mid-mitotic resting stages" in the opalinids as described by other investigators are misinterpretations. (Nucleoli in the resting nucleus were considered as chromosomes, while the chromatin reticulum was overlooked by them.)

Chen, 1937, described a new method for preserving and shipping smears of opalinids.

Carini, 1937, described seven new species of opalinids found in Brazil: *Opalina faber* in *Hyla faber; O. elongata* in *Hyla faber; O. nebulosa* in *Hyla nebulosa; O. rugosa* in *Hyla nebulosa; O. rubra* in *Hyla rubra; O. raddiana* in *Hyla raddiana;* and *O. mogyana* in *Hyla leucophyllata.* [The descriptions of some of these species are too scant to allow specific identification.]

Horning, 1937, reported some experimental studies on the cytoplasmic inclusions of opalinids. In addition to the Golgi bodies that Richardson and Horning (1931) described, Horning distinguishes two principal cytoplasmic components: the mitochondria and the vegetative granules of the endoplasm. The mitochondria react to experimental conditions such as alterations in the pH of the external medium and cellular injury. Under the influence of radium radiations the mitochondria are re-oriented so that they assume a transverse polarity to the longitudinal axis of the organism. Later the mitochondria are segregated by the radiations so that they lie apart from the vegetative granules with which they are closely associated in the normal organism.

Lavier, 1937, reported the absorption of bile pigments by the trophozoites and cysts of opalinids. The bile pigments may be deposited in the opalinids in the form of brownish-red crystals.

Sandon, 1938, reported the presence of *Zelleriella* in South Africa. *Z.* (*africana* A) and *Z.* (*africana* B) were found in the rectum of two species of *Rana* from the neighborhood of Capetown.

Carini, 1938b, revised the genus *Zelleriella* inhabiting *Leptodactylus ocellatus* in Brazil.

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